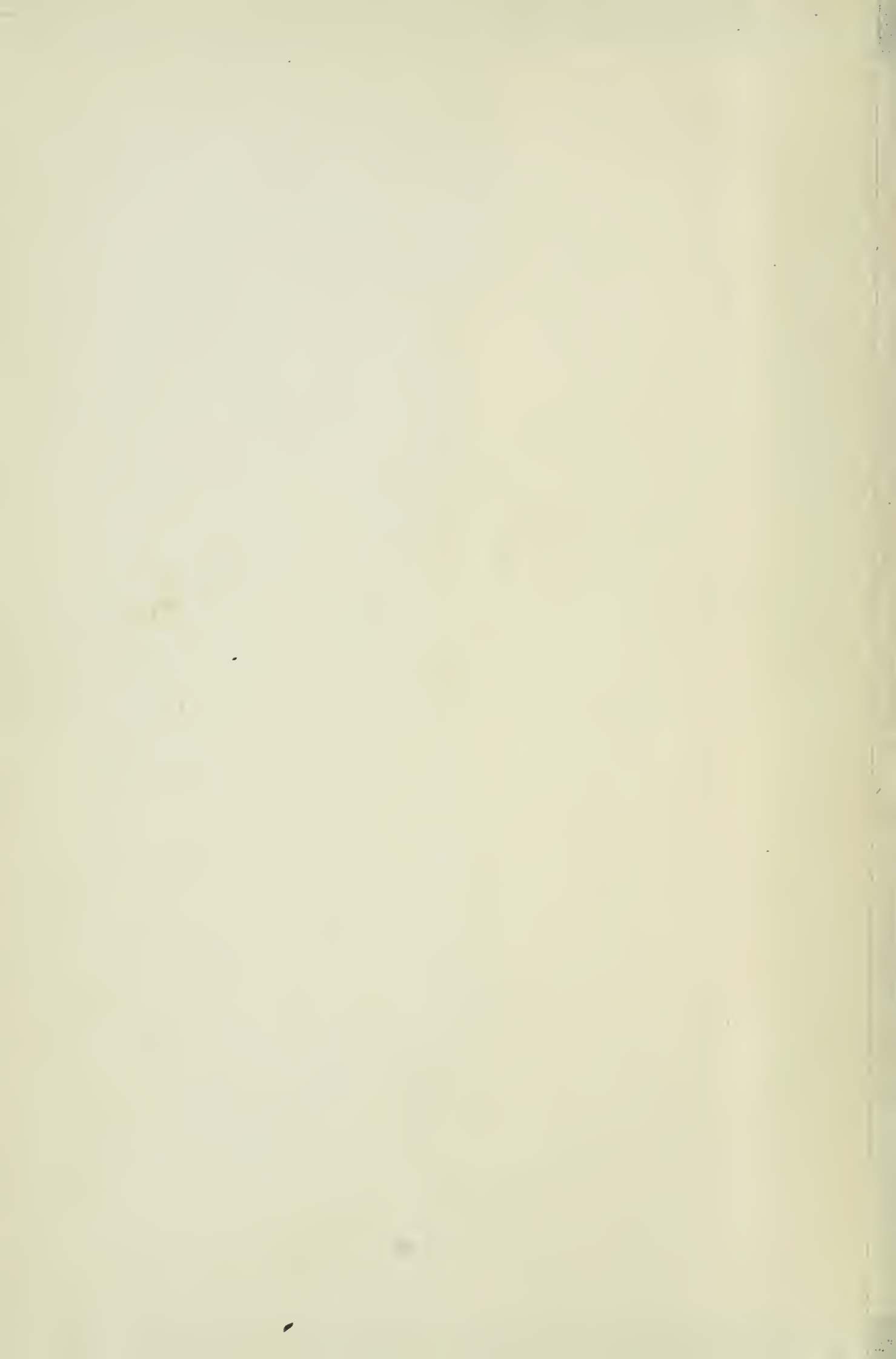


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A TOXICOLOGICAL STUDY OF SOME ALCOHOLS, WITH ESPECIAL REFERENCE TO ISOMERS

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From the Pharmacological Laboratory, the Johns Hopkins University

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The comparative pharmacological, and, more particularly, toxicological, properties of various alcohols have been the subject of investigations on the part of many authors. The work on the subject has, for the most part, been confined to the first five members of the aliphatic series, and almost entirely to the primary alcohols in the cases in which several isomers are known. The most significant and important observation on the comparative pharmacology of these alcohols was made as early as 1869. Richardson, in 1869, stated, in the *Medical Times and Gazette*, volume 2, page 705, that the toxicity of the alcohols belonging to the fatty acid series increased in proportion to their molecular weight. This observation, known as Richardson's law, has been confirmed and amplified by all the later observers. Among the most important investigators along these lines may be mentioned Dujardin-Baumetz (1), Joffroy and Servaux (2), Picaud (3), and Baer (4), who studied the comparative lethal doses; while Efron (5), Schneegans and v. Mering (6), Breyer (7), Dold (8), Kuno (9) and others investigated the effects of the various alcohols on special organs.

Dujardin-Baumetz and Audige found the toxicity of ethyl, propyl, butyl and amyl alcohols to be in the ratio of 1.0, 2.0, 4.2 and 5.2, respectively. According to these authors, the toxicity of methyl alcohol was found to be about the same or slightly higher than that of ethyl. Most authors, however, find that in acute experiments methyl alcohol is less poisonous than ethyl alcohol. Thus, Joffroy found the relation between

the lethal doses of alcohol—methyl, ethyl, propyl, butyl and amyl—to be expressed by the ratios 0.46, 1.0, 3.5, 8.0 and 18.5. Picaud gives the following figures for the same relationship: 0.66, 1.0, 1.0, 3.0 and 16.0. According to Baer, the ratios are as follows: 0.8, 1.0, 2.0, 3.0 and 4.0. It will be noted that according to the last three authors, the toxicity of the various alcohols increases in proportion to their molecular weight. The actual figures obtained by various observers are different, according to the methods of administration. Some injected the drugs intravenously, which is, on the whole, the most satisfactory method

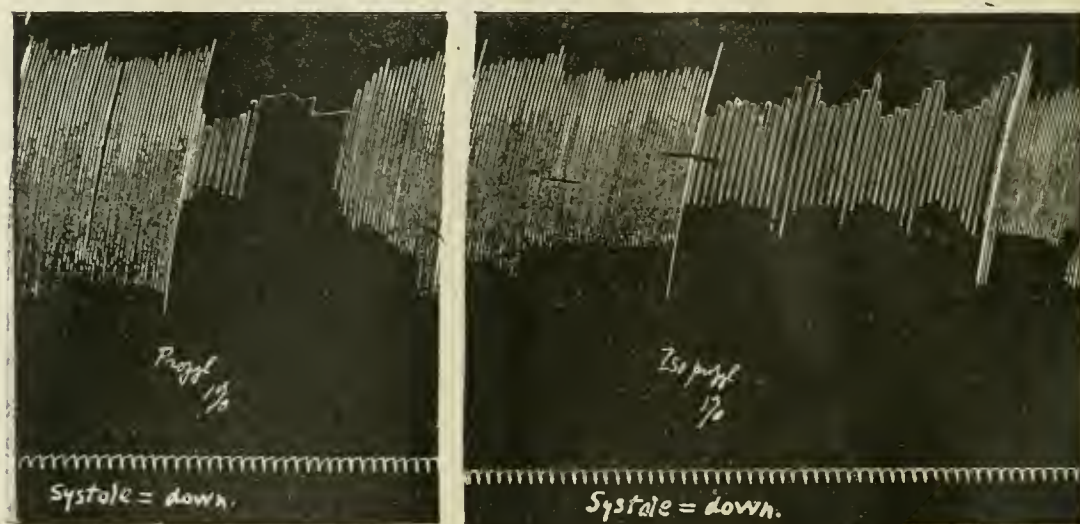


FIG. 1. FROG'S HEART SUSPENSION PREPARATION

A, Perfusion with propyl alcohol 1 per cent in Locke; B, perfusion with isopropyl alcohol 1 per cent in Locke.

of studying the lethal doses for acute toxic experiments. This is the method, as is well known, now employed for standardizing the digitalis bodies. Dujardin-Baumetz found the same relationship to hold good after administering the alcohols by stomach.

Efron (1885) studied the pharmacological action of the various alcohols on nerve and muscle. This author found that Richardson's law applies also to the behavior of these organs. All the alcohols produce a more or less marked primary excitation, followed by paralysis, and the relative toxicity increases with the molecular weight. Schneegans and v. Mering found the

narcotic action of the various alcohols to follow more or less Richardson's law. Picaud studied the effects of various alcohols on fishes, amphibians and other animals. Dold studied the comparative effects of the various alcohols on the frog's heart, while Hermeter investigated the effects of the same on the mammalian heart. All these authors found a relationship between the toxicities to be very much in accordance with Richardson's earlier observations. Further work by Kuno on mam-

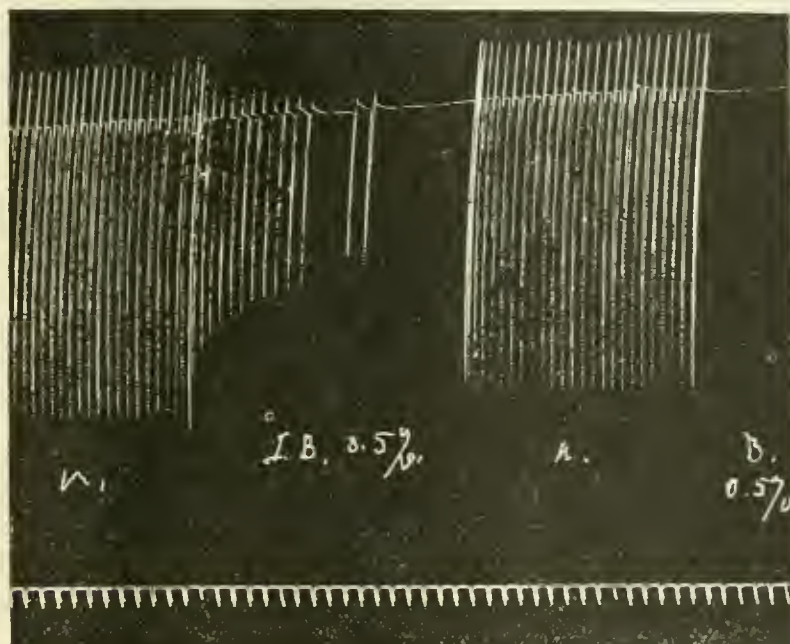


FIG. 2. FROG'S HEART SUSPENSION PREPARATION

N, Normal contractions; systole, down; *I.B.*, isobutyl alcohol 0.5 per cent; *B*, butyl alcohol 0.5 per cent.

malian hearts, by Breyer on motor nerves and ciliated epithelium, by Fühner (10) on the eggs of the sea-urchin, by Blumenthal (11), and by Verzar (12), all corroborated, broadly speaking, the original law enunciated by Richardson. The chief exception noted was that by Blumenthal, who claimed that methyl alcohol is more poisonous than ethyl. Many other interesting pharmacological facts concerning the various alcohols may be found in Professor Abel's monograph on the subject (13).

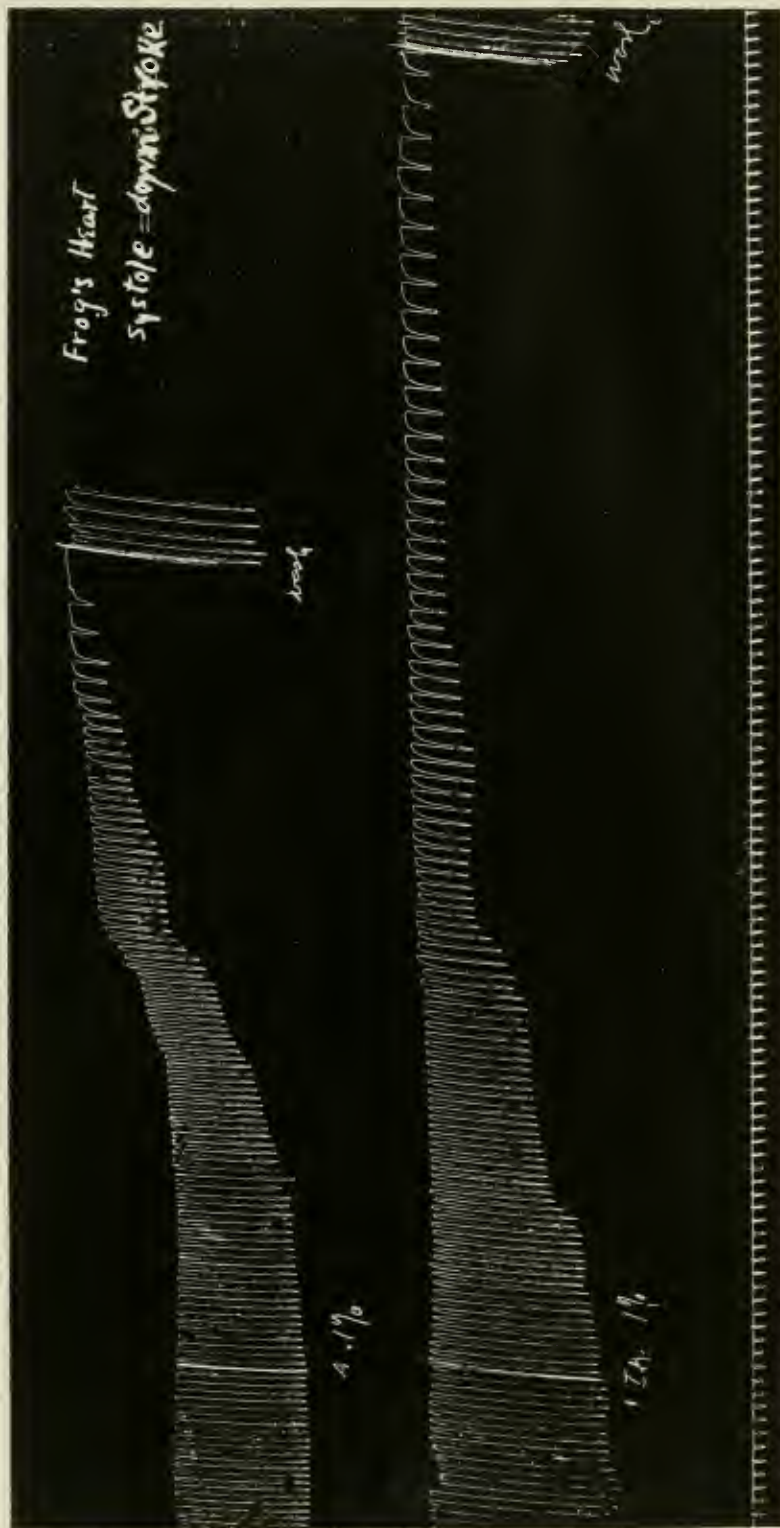


FIG. 3. FROG'S HEART SUSPENSION PREPARATION

A, Perfusion with amyl alcohol 0.1 per cent; I.A., perfusion with isoamyl alcohol 0.1 per cent.

While the comparative physiological effects of the primary, or normal, alcohols have been studied extensively, very little, indeed, is known concerning the pharmacological action of the secondary alcohols. The present author became interested in this subject in connection with a study of isopropyl alcohol, which he has undertaken at the suggestion of Prof. Ira Remsen. In order to obtain a comprehensive idea of the toxicological effects of isopropyl alcohol, the author made experiments with primary alcohols, on the one hand, and secondary alcohols, on the other. The compounds studied were the following: Methyl alcohol, ethyl alcohol, propyl alcohol, isopropyl alcohol, butyl alcohol, isobutyl alcohol, amyl alcohol, isoamyl alcohol, and, to some extent also, benzyl alcohol.

The first investigation undertaken by the author was to determine the lethal doses of the various alcohols. For this purpose the cat method was employed. This method, which is extensively followed for the testing of various drugs, is now recognized as the most convenient method of assaying digitalis and other heart drugs. It consists, briefly, in injecting into the vein of a cat, under light ether anesthesia, at regular intervals of time, a solution of the drug to be tested. The amount of the drug necessary to kill the cat, expressed in terms of its weight, is the lethal dose. In performing the experiment, care is taken to note the form of death produced—whether through paralysis of the heart, paralysis of the respiratory center, etc.

It was found, on testing methyl, ethyl, propyl, butyl, and amyl alcohols, that their toxicity increased, broadly speaking, with their molecular weight. In other words, Richardson's law was found to hold good. In the experiments performed, 1 and 5 per cent solution of the various alcohols were made in physiological saline, and injected rather rapidly, but at regular intervals of time (2 cc. per minute). The figures obtained are shown in the subjoined table. It will be noted that while the absolute fatal doses may be different from those obtained by other authors (due to the rapidity of administration and concentration of the solution), Richardson's law was confirmed completely.

Following the injections of the primary alcohols just mentioned, the lethal dosage of the secondary alcohols was determined by the same method, and the figures compared with those obtained in case of the normal propyl, butyl and amyl alcohols. It was found, in case of the three groups of isomers, that the primary or normal alcohols were always more toxic than the secondary, or the isopropyl, isobutyl and isoamyl alcohols.

After studying the lethal dosage of the various compounds mentioned above, a comparative study of their solutions on the isolated frog's heart was undertaken. It was interesting to find that on this isolated organ Richardson's law held good, and,

TABLE I

ALCOHOL	CONCENTRATION INJECTED	LETHAL DOSE PER KILO WEIGHT	LETHAL DOSE PER KILO IN TERMS OF THE PURE ALCOHOL	RATIO IN TERMS OF ETHYL ALCOHOL
	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	
Methyl.....	5	118.0	5.9	0.8
Ethyl.....	5	100.0	5.0	1.0
Propyl.....	5	40.0	2.0	2.5
Butyl.....	5	6.0	0.3	16.6
Amyl.....	1	14.8	0.15	33.2
Isopropyl.....	5	5.0	2.5	2.0
Isobutyl.....	5	18.0	0.9	5.5
Isoamyl.....	1	26.0	0.26	19.2
Benzyl.....	1	60.0	0.60	8.3

furthermore, that in every case the normal alcohols were more toxic than the secondary ones, as illustrated by the curves.

In order to test the curious relationship between the normal and secondary alcohols further, observations were made on their action on isolated plain muscle. It was found that in case of the ureter, which, in many respects, as has been shown by the author elsewhere, is the most convenient smooth muscle organ for quantitative comparisons, the same relationship held good (14). In every case the primary alcohol paralyzed the smooth muscle more quickly than the secondary ones.

Inasmuch as the author was especially interested in the properties of isopropyl alcohol, some special studies were made

with that compound. The toxicity of isopropyl alcohol through inhalation was compared with that of methyl and ethyl alcohols. This was studied on rats. The animals were confined in a chamber containing an open vessel, suitably screened off, filled with the above alcohols, so that the air of the chamber was saturated with their fumes. It was found that methyl alcohol quickly killed the rats, the latter succumbing in a day or two.

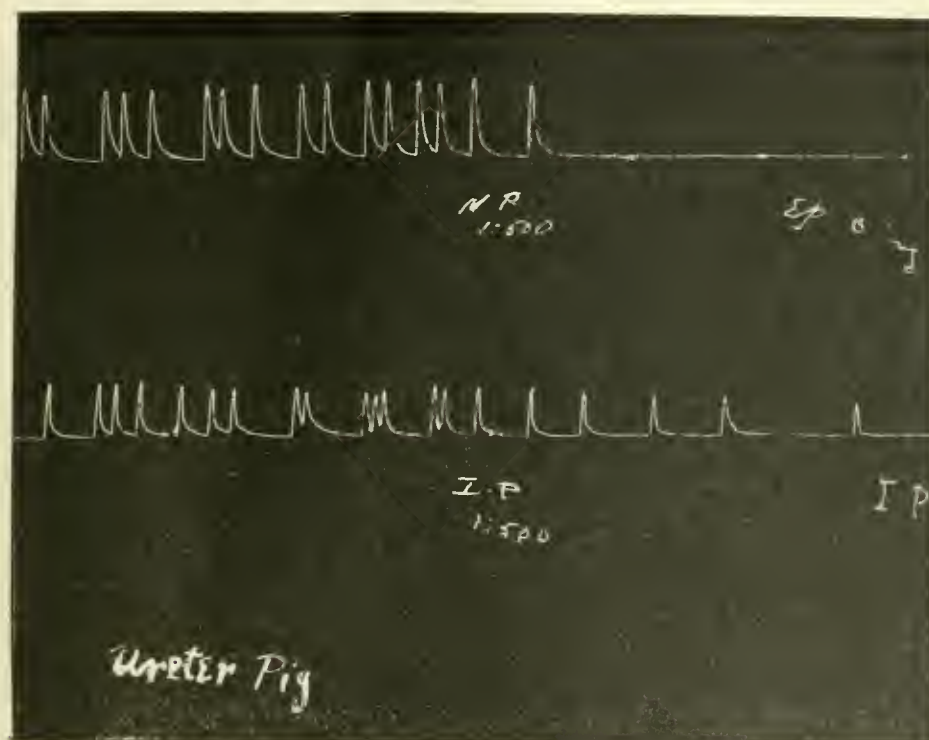


FIG. 4. PIG'S URETER

N.P., Normal propyl alcohol 1:500; *I.P.*, isopropyl alcohol 1:500

Ethyl alcohol proved also fatal to the animals, after exposure to its fumes for several days in a warm room. Rats confined in a chamber containing an open vessel with isopropyl alcohol, in a warm room, were very little affected, and were found to be apparently in normal health after a week's exposure. No blindness or defects in vision were noted after exposure of the rats to the fumes of isopropyl alcohol in these experiments.

The peculiar relationship between the primary and secondary alcohols noted by the author has not been described elsewhere.

It is interesting to note, however, that the properties of one of the isomers, namely, isopropyl alcohol, have been investigated by Efron. That author found that isopropyl alcohol is less depressant than the normal propyl alcohol for nerve fibers, and his observations harmonize with the findings of the present investigation.

It will be seen that a comparative study of the various alcohols reveals the fact, on the one hand, that their toxicity increases

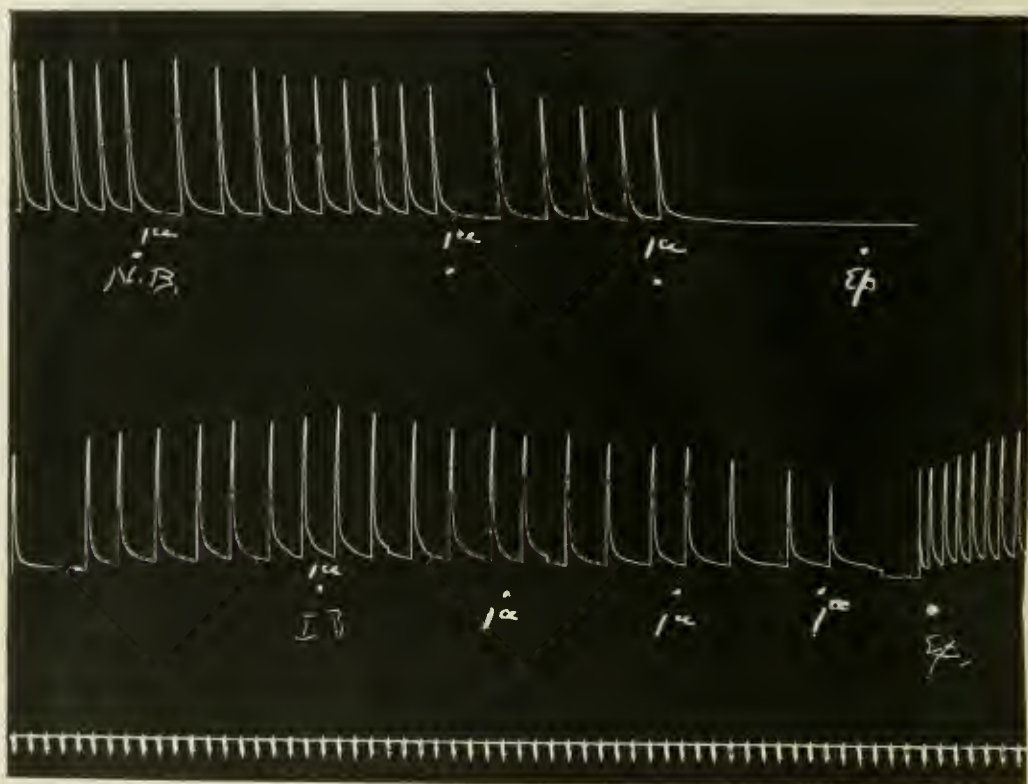


FIG. 5. PIG'S URETER

N.B., Normal butyl alcohol 1 per cent, paralyzed after three doses 1 cc. each in 30 cc. Locke. No response to epinephrin after that. *I.B.*, Isobutyl alcohol 1 per cent, contraction inhibited after 4 cc. but still revived by epinephrin.

with their molecular weight, and, on the other hand, that the normal propyl, butyl and amyl alcohols are more toxic than the isopropyl, isobutyl and isoamyl alcohols, as revealed by studies of the killing dose for cats, by experiments on isolated frog hearts, and by observations on the contractions of isolated plain muscle.

Furthermore, it should be noted that the lethal doses obtained in this investigation are those for acute intoxications. This is important to bear in mind in connection, especially, with wood alcohol. Methyl alcohol is known to be extremely poisonous, and is regarded as a more violent poison than grain alcohol. This is true only in respect to its remote effects, as commonly seen when it is taken by mouth. When introduced into the alimentary canal, wood alcohol acts not as such, but exerts its most deleterious effects through its decomposition products,

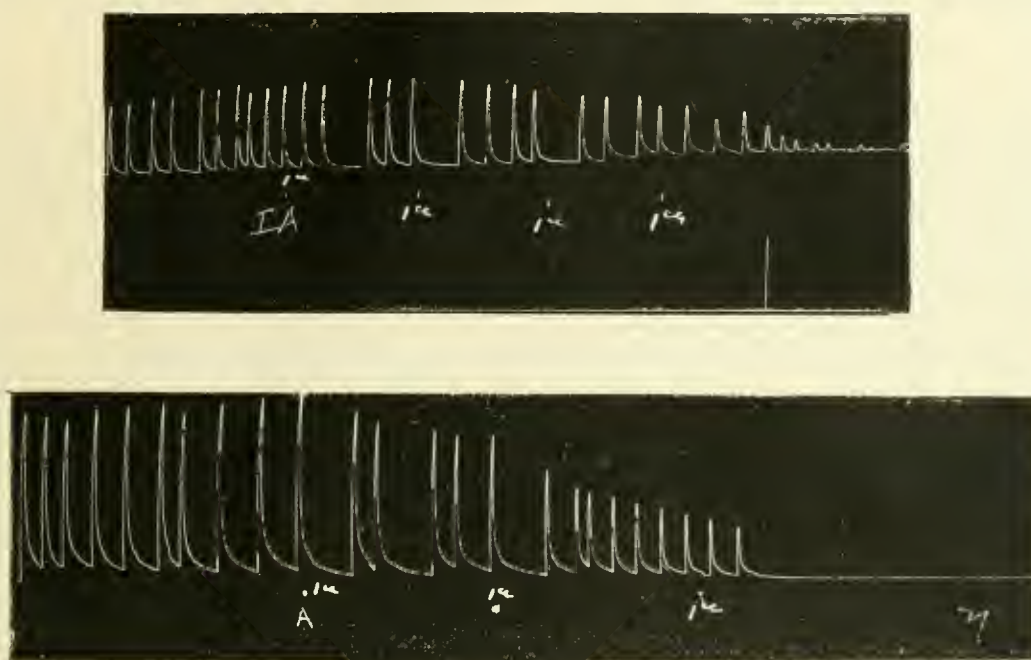


FIG. 6. PIG'S URETER

A., Amyl alcohol 1 per cent; I.A., isoamyl alcohol 1 per cent

especially formaldehyde and formic acid. It is these secondary products that render wood alcohol more poisonous than ethyl alcohol, when taken by mouth. When injected into the vein, however, and acting acutely upon the heart and the brain, methyl alcohol is considerably less toxic than ethyl alcohol.

It is interesting to compare this difference between the immediate and remote or indirect effects of methyl alcohol with the immediate and secondary, or remote, effects of benzyl alcohol. Benzyl alcohol, which the present author has shown to be a

valuable local anesthetic, is a comparatively little toxic drug (15). The lethal dose of benzyl alcohol, as obtained by intravenous injections of its solutions, is, however, very much greater than the toxic dose of it, when administered even in pure form and undiluted by mouth. Indeed, the author has not been able to kill dogs by administering even large quantities of benzyl alcohol through the stomach tube. The difference between the intravenous and oral administrations of the drug is due to the metabolism of benzyl alcohol and its excretion in the form of hippuric acid, when taken by mouth.

SUMMARY

1. The toxicity of the normal alcohols—methyl, ethyl, butyl and amyl—increases with their place in the aliphatic series, as indicated by the lethal dosage for cats, and by their effects on isolated frogs' hearts and plain muscle preparations.

2. The secondary propyl, butyl and amyl alcohols were found to be less toxic than the corresponding primary alcohols.

3. In discussing the toxicity of alcohols, a distinction should be made between the acute or immediate, and the secondary or remote effects of the drugs, as is well illustrated by the studies on methyl and benzyl alcohols.

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THE ELIMINATION OF CARBON MONOXIDE FROM THE BLOOD AFTER A DANGEROUS DEGREE OF ASPHYXIATION, AND A THERAPY FOR ACCELERATING THE ELIMINATION

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Within a few hours after profound but not fatal poisoning with carbon monoxide no trace of the gas is found in the blood (1). It has been eliminated through a reversal of the process through which it was absorbed. And yet for days, months, or even for life, structural degenerations, and functional derangement, usually either nervous or cardiac, may continue.

No therapeutic measures (2) as yet suggested have proved of value, or promise much, in relieving these effects. They are the sequelae of the injury wrought by oxygen deficiency and its concomitants while the gas was still in the victim's blood.

Our attention happened to be directed to the question of the rate at which the gas is eliminated. We thus came upon a point which has been overlooked, although it is obvious enough when once seen. It suggests a therapy—or perhaps rather a prophylaxis.

So far as we are aware, no one has devoted particular attention to the rate at which carbon monoxide is eliminated during the first hour or two after a gassing which has produced a saturation of the blood of considerably more than fifty per cent, with coma and its accompaniments.

We have recently studied among other questions concerning carbon monoxide the rate of elimination in men who have reached saturations of 20 or 30 per cent (3). Haldane (4) has recorded the rate of elimination after saturations of 40 or even 50 per cent.

All of these data indicate an elimination during the first hour of about one-half of the amount previously absorbed.

But these observations do not touch the matter to which we have here to call attention; for the subjects in all of these experiments were breathing practically normal volumes of air, and their metabolism—oxygen consumption and CO_2 production—was at most only slightly impaired. It should be mentioned however that Haldane (4) noted that when carbon monoxide asphyxiation becomes acute over-breathing occurs; and that in coma the body rapidly becomes cold, indicating depressed oxidation. He has demonstrated also in numerous papers the close dependence of volume of breathing upon the amount of CO_2 requiring elimination from the body.

No one however has made the obvious inference from these facts. The observations which we have to report came to us as a purely experimental and unanticipated discovery.

DEPRESSED BREATHING AND CONTINUED ASPHYXIA

In brief, our observations indicate that during the development of asphyxia under carbon monoxide there is excessive breathing which markedly reduces the body's store of CO_2 . Under asphyxia the oxidative metabolism and production of CO_2 are apparently greatly depressed. When the victim is removed from the poisonous atmosphere the breathing, lacking its normal stimulus, remains at a very low level for an hour or more. The body lacks the CO_2 needed to maintain an efficient ventilation of the lungs. The elimination of carbon monoxide during this time is therefore very slight. Although the body is surrounded by fresh air the condition of asphyxiation continues within the tissues. Even the administration of oxygen has no great effect, for it is not adequately inhaled. Until a sufficient amount of CO_2 is reaccumulated in the blood, and a sufficient oxidative metabolism recovered, so that efficient breathing is restored, the injurious conditions within the living cells of the nervous system and heart are only very gradually abated. *When, as in our experiments, the period of gassing is brief but acute, most of the*

asphyxia during which the harm is wrought may occur after the subject is removed from the poisonous atmosphere. It may be largely during this time that the autolytic and necrotic processes are so intensified as to be thereafter irreversible.

This is probably what happens sometimes, for example, in the case of city firemen overcome by smoke. It is likely to occur also in acute industrial poisoning around gas plants and blast furnaces. Even in the common sleeping room accident in which the gassing progresses over night, the period of most profound asphyxia comes at the end and is continued by the depressed breathing for a considerable time after the victim is removed to fresh air. In such cases the abbreviation of the post-gassing period of depressed breathing and its continued asphyxia may be of critical importance in preventing permanent damage.

The treatment which, as we shall show, achieves the rapid termination of asphyxia is the inhalation of a mixture of oxygen and CO_2 . The CO_2 induces active respiration which affords the oxygen an opportunity to displace carbon monoxide rapidly. It is probably of value also in replacing in the blood and tissues the CO_2 lost by the previous over breathing (5).

EXPERIMENTAL PROCEDURES

Our experiments were performed upon dogs. A sample of blood was obtained from the femoral artery under local anesthesia with cocaine. The animal was then placed in one of the gassing chambers developed here for the experiments done under the Bureau of Mines and the Chemical Warfare Service (6). A sufficient amount of city illuminating gas was added to the air in a large gasometer to produce a concentration of 0.3 to 0.4 per cent of carbon monoxide. This mixture was then passed into the chamber by a motor driven air blower.

During the gassing the manifestations presented by the animals were almost identical in every instance. Up to unconsciousness the picture presented resembled closely that of slow anesthesia with ether. At first the animal moved about restlessly and scratched at the glass sides. A stage of excitement followed;

then muscular weakness. The animal's hind legs relaxed, and it supported itself with wide spread front legs. At about this time the respiration became more and more vigorous, culminating in violent and prolonged hyperpnea. Saliva dripped from the jaws and vomiting usually occurred. The animal then lay down, and there was thereafter only an occasional feeble movement. The breathing became of the Cheyne-Stokes type, and unconsciousness followed.

After five or ten minutes of unconsciousness the animal was removed from the gas chamber. A sample of arterial blood was at once taken. In this the percentage saturation of the blood with carbon monoxide was determined by the Haldane method (7); a dilution of the original normal blood was titrated with a dilute solution of carmine until equality of tint with the blood containing carbon monoxide was obtained. At suitable intervals thereafter additional blood samples were taken from the animal and the percentage saturation with carbon monoxide determined. At the same time the general physiological condition of the animal was recorded.

Although we here speak of these as experiments on carbon monoxide poisoning, it should be noted that illuminating gas was used. In some experiments performed to determine the relative toxicity of illuminating gas and of pure carbon monoxide, made from formic acid, we found that with the former death occurred when the concentration of carbon monoxide in the blood reached about 65 per cent saturation, while with the pure gas in equal dilution death occurred at blood saturations of about 85 per cent. Evidently the illuminating gas used owes about 25 per cent of its toxicity to some substance other than carbon monoxide. But as this substance appears to be likewise directly or indirectly a powerful respiratory stimulant, and as illuminating gas therefore induces even more marked over breathing than is the case with pure carbon monoxide in corresponding dilutions, the validity of the conclusions to be drawn from the experiments is not affected.

THE ELIMINATION OF CARBON MONOXIDE IN UNTREATED ANIMALS

Three experiments were performed in which, after a gassing of about half an hour, the animal was merely removed from the chamber, and the course of events observed. One died in thirty minutes. Owing to the inefficient breathing, there was a scarcely measurable decrease in the concentration of carbon monoxide in its blood. The other two exhibited during the first half hour or more a markedly depressed respiration; and there was a correspondingly slight decrease in the concentration of carbon monoxide in the blood. Thereafter the volume of breathing gradually

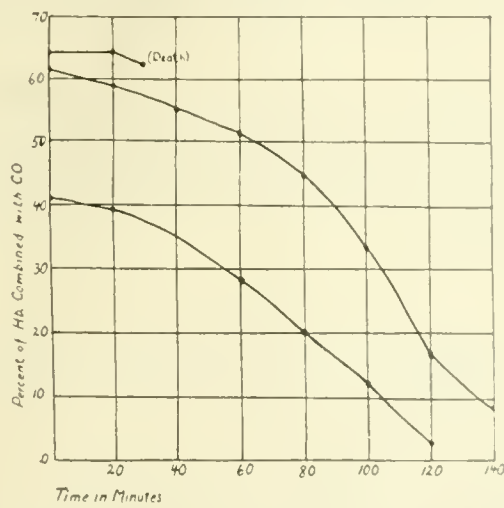


FIG. 1. PLOTTED FROM THE DATA OF EXPERIMENTS 1, 2, AND 3

Showing the rate of elimination of carbon monoxide from the blood of profoundly asphyxiated but thereafter untreated animals.

increased, and the elimination of carbon monoxide progressed correspondingly. Consciousness returned slowly after about an hour; but even after several hours the animals were still sick and unsteady.

The analytical data are plotted in figure 1 from the following protocols:

Experiment 1. Dog, male, 7.5 kilos. Gassed for thirty minutes. Completely unconscious on removal from gassing chamber.

The times at which blood samples were taken and the percentage saturation with carbon monoxide found were as follows:

Time, minutes.....	0	20	60	80	100	120
Percent CO.....	41	39	28	20	12	4

Experiment 2. Dog, male, 10 kilos. Gassed for thirty-five minutes. Completely unconscious. Blood samples and analyses as in previous experiment.

Time, minutes.....	0	20	40	60	80	100	120	140
Percent CO.....	62	58	55	52	45	33	16	0

Experiment 3. Dog. Female, 6 kilos. Gassed for thirty minutes. Completely unconscious. Death thirty minutes later.

Time, minutes.....	0	20	30
Percent CO.....	64	62	62

THE ELIMINATION OF CARBON MONOXIDE UNDER INHALATION OF OXYGEN

The procedure in these experiments was similar to that in the preceding experiments except that the animals were made to inspire pure oxygen by means of a mask and valves with as little rebreathing as possible.

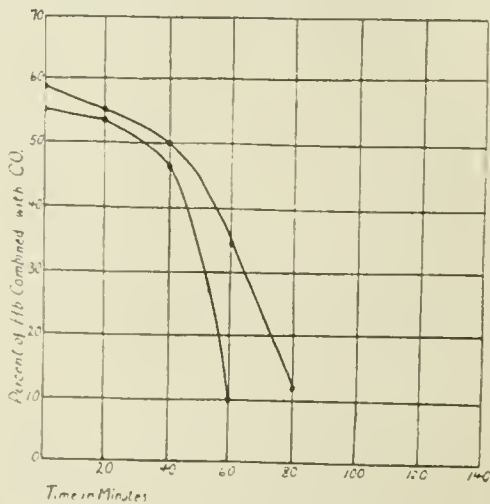


FIG. 2. PLOTTED FROM THE DATA OF EXPERIMENTS 4 AND 5

Showing the rate of elimination of carbon monoxide from the blood of animals under inhalation of oxygen.

It will be seen from the protocols and from figure 2, in which the results are plotted, that during the first forty minutes the rate of elimination of carbon monoxide was only slightly more rapid than in the case of animals breathing merely air. This was manifestly due to the marked depression of breathing during

this time. During the next forty minutes, however, the rate of elimination of carbon monoxide was markedly accelerted by oxygen inhalation. The subsequent condition of the animals was distinctly better than was the case with untreated animals.

Experiment 4. Dog, male, 9 kilos. Gassed thirty-four minutes. Completely unconscious. Blood samples and analyses as in previous experiments.

Time, minutes.....	0	20	40	60	80
Percent CO.....	58	55	50	33	12

Experiment 5. Dog, female, 7 kilos. Gassed thirty-eight minutes. Completely unconscious.

Time, minutes.....	0	20	40	80
Percent CO.....	55	53	57	10

The third figure, 57, involves probably some degree of analytical error, but it is given as found.

THE ELIMINATION OF CARBON MONOXIDE UNDER INHALATION OF MODERATE PERCENTAGES OF CO₂ IN AIR

The procedures in these experiments were similar to the preceding except that a mixture of CO₂ and air was supplied under a very slight pressure to a funnel fitting loosely over the animal's nose. The expired air passed out around the sides of the mask. The air was delivered by a blower. The CO₂ came from a tank of compressed gas. The rate of flow of the air and the concentration of CO₂ were regulated by means of a double overflow flow meter (8). The percentage of CO₂ was determined by analysis.

The results of these experiments are shown in the following protocols and are plotted in figure 3. They indicate that the marked stimulation of breathing induced by the inspired CO₂ resulted in a very much more rapid rate of elimination of carbon monoxide from the blood and return of consciousness than in either of the previous two sets of experiments. The subsequent condition of the animals was correspondingly better.

Experiment 6. Dog, male, 10 kilos. Gassed for thirty minutes. Completely unconscious. After removal from the chamber the animal was allowed to breathe air containing 10 per cent CO₂. The volume of breathing was very greatly augmented. Blood samples were taken and analyses made as in previous experiments.

Time, minutes.....	0	15	30
Percent CO.....	49	36	8

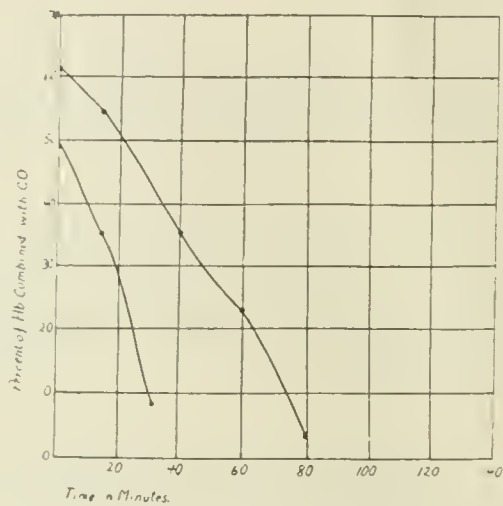


FIG. 3. PLOTTED FROM THE DATA OF EXPERIMENTS 6 AND 7

Showing the rate of elimination of carbon monoxide from the blood of animals while breathing air plus CO₂.

Experiment 7. Dog, female, 6.5 kilos. Gassed for thirty minutes. Completely unconscious. After removal from the chamber the animal was allowed to breathe air containing 6 per cent CO₂. Respiration moderately augmented.

Time, minutes.....	0	15	40	60	80
Percent CO.....	61	54	36	24	4

THE ELIMINATION OF CARBON MONOXIDE UNDER OXYGEN PLUS
CO₂

In these experiments the animals were gassed fully as deeply as in any of the preceding. They were completely comatose on removal from the chamber. After the usual blood sample had been taken, they were made to inhale oxygen to which 10 per

cent of CO₂ had been added. The inhalation was administered through a mask and double valves so as to avoid any rebreathing.

A vigorous respiratory response occurred almost at once. Within five minutes the animals were conscious and struggling. When the inhalations were terminated at the end of twenty-two and thirty minutes respectively, the subjects exhibited almost complete restoration to normal condition. The data of the experiments are given in the following protocols and are plotted in figure 4.

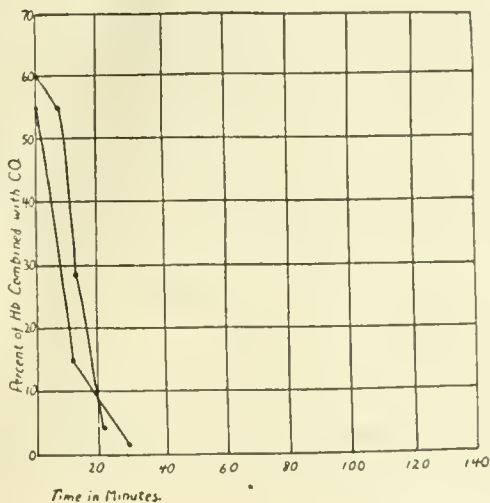


FIG. 4. PLOTTED FROM THE DATA OF EXPERIMENTS 8 AND 9

Showing the rate of elimination of carbon monoxide from the blood of animals under inhalation of oxygen plus CO₂.

Experiment 8. Dog, male, 14 kilos. Gassed for thirty-seven minutes. Completely unconscious. After removal from the chamber the animal was allowed to breathe oxygen and 10 per cent CO₂. The volume of breathing was immediately very greatly augmented and consciousness rapidly returned.

Time, minutes.....	0	15	30
Percent CO.....	55	15	2

Experiment 9. Dog, male, 12 kilos. Gassed for forty minutes. Completely unconscious. Treated as in previous experiment with similar results.

Time, minutes.....	0	8	15	22
Percent CO.....	60	55	28	4

CONCLUSIONS

It is here shown that during the development of carbon monoxide asphyxia there is vigorous hyperpnea, and that thereafter, probably owing to deficient oxygenation and other causes, there is a diminished production of CO_2 . As a result of deficiency of CO_2 in the blood, asphyxiated animals when restored to pure air exhibit for half an hour or more a very marked depression of breathing. The rate of elimination of carbon monoxide is correspondingly slow. The condition of tissue asphyxia is thus continued, although the body is surrounded by fresh air.

It is suggested that this post-gassing period of continued asphyxia may be of critical importance in inducing subsequent structural degenerations and functional impairments. Its abbreviation is therefore an important object both for therapy and prophylaxis.

Oxygen inhalation during this period has only a slight effect; it is not adequately inspired.

Inhalation of CO_2 diluted with air has an immediate effect. It augments breathing and thus hastens the elimination of carbon monoxide.

Inhalation of oxygen plus CO_2 is far more effective than either gas alone; for the augmented breathing allows the oxygen to effect a rapid displacement of carbon monoxide from the blood. Functional restoration is correspondingly accelerated.

We hope to carry this matter on into practical application.

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THE EFFECT OF EMETINE ON ENTAMOEBA HISTOLYTICA, IN STOOLS

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Emetine was originally used in the treatment of amoebic dysentery by Rogers (1) because Vedder (2) had shown that emetine in dilutions up to 1:100,000 would kill free-living amoebae in broth cultures in twenty-four hours. Rogers stated that *Entamoeba histolytica* in bits of mucus in stools, placed in normal salt solutions of emetine hydrochlorid are immediately killed in dilutions of 1:10,000, and "after a few minutes they are rendered inactive and apparently killed by as weak a solution as 1:100,000."

Lyons (3), working on the same problem at the same time with ipecac of uncertain strength, failed to obtain amoebacidal action *in vitro*.

Wherry (4) used broth cultures of free-living amoebae and found emetine lethal at times in dilutions of 1:20,000, but at other times this dilution was harmless.

Kuenen and Swellengrebel (5) applied emetine directly to *Entamoebae* in the stools, finding 1:10,000 generally ineffective, and 1:5000 frequently so.

Rogers (6) finding work on *Entamoebae* in stools uncertain, turned to *Paramecia*. He found that emetine in dilutions of 1:200,000 killed these ciliates in one hour.

Kolmer and Smith (7) working with *Entamoeba gingivalis*, Gros, found that emetine in dilutions up to 1:1280 stopped all motion within one hour, but point out that the results are modified by the amount of organic matter, particularly pus, present.

Pyman and Wenyon (8) found that the growth of *Amoeba limax* on agar impregnated with emetine was inhibited by a strength of 1:10,000.

Walters, Baker and Koch (9) conclude that "emetine hydrochlorid in solution of 1:1000 when acting on water amoebae for one hour, or in solution of 1:5000 acting for three hours, destroyed many of these organisms, but was not uniformly amoebacidal," and that, "emetine hydrochlorid in solutions as strong as 1:100 are not rapidly destructive to *Entamoeba buccalis*."

Dale and Dobell (10) tested the effect of emetine on emulsions of *Entamoeba histolytica* from the gut scrapings of kittens experimentally infected from man. Using strain "A," dilutions of 1:1000 were destructive in one hour, in about half of their experiments. Strain "B" was not affected by 1:100 in thirty minutes.

After using emetine clinically for about a year the writer became skeptical of the action of the drug on *Entamoebae* in any such dilutions as 1:100,000 and in the winter of 1913-1914 carried out a number of tests to determine the strength of emetine necessary to kill these organisms in fresh human stools. However, while these tests were being made, Rogers (6) published his work on the protozoacidal action of emetine in which he claimed that *Paramecia* were killed by 1:500,000 emetine in three hours, and by 1:1,000,000 emetine in twenty hours. At the same time Vedder (11), citing Wherry's destruction of amoebae by 1:200,000 emetine in twenty-three and one-half hours, said that, "since the parasitic amebas are far less resistant to unfavorable agencies, it was reasonable to suppose that these also would be destroyed by emetine."

As the writer could not get emetine 1:1000 to kill *Entamoebae* in stools with any regularity, these observations were discontinued, since experiments with cultural free-living protozoa seemed to be less open to objection. In view of the later work of Dale and Dobell, this bit of evidence is now offered, as few observations of the action of emetine applied directly to *Entamoebae* in the stools have been recorded.

All observers have been confronted with the difficulties in determining the lethal strength of emetine solutions for *Entamoebae* when applied directly. (1) This obligatory parasite dies rather soon after leaving the body, so that the element of

time, considered so important in the action of emetine, cannot be properly taken into account. (2) The death or fatal injury of the organism is hard to determine without disintegration. (3) The organism cannot be cultivated. (4) The alkaloid may combine with other organic matter in the stools and become inert. (5) Entamoebae buried in mucus are well protected against water or normal salt solutions of emetine.

These difficulties have led to the use of cultural amoebae that can be exposed to the action of emetine for any length of time, or to the use of free-living ciliates. To determine the death of the organism the fact that eosin will penetrate and stain dead cells has been utilized, or the Entamoebae, after exposure to the emetine, have been injected into kittens. No one seems to have repeated James' (12) work on staining emetine-injured Entamoebae after direct application of the drug. The use of broth cultures would seem to be open to the same objections as stools in regard to the fixation of emetine by organic matter. The use of ciliates seems now of doubtful value, as Dale and Dobell have shown that substances highly toxic for *Paramecia*, such as cusparine hydrochlorid, may have no effect at all on *Entamoeba histolytica*. And these observers have further shown that, aside from differences in genus and species, "even in the same emulsion it was evident that individual amoebae differed widely in their resistance, so that an exact determination of lethal concentration was out of the question." But in spite of these difficulties, all observers, except Rogers, using various organisms and various methods have obtained approximately the same results.

The applications of emetine here recorded were made by putting a small loop of feces under a coverglass, making sure that the specimen was full of active Entamoebae, then removing the coverglass and mixing the feces thoroughly with about five times its volume of warm emetine solution, thus floating up the coverglass. The emetine was dissolved in normal salt solution except in the case of *Entamoeba* "C. S.," when distilled water was used. During observation a new supply of emetine was frequently added to overcome evaporation; at times the

mixtures were re-stirred to liberate the organisms more completely from mucus or pus. The slides were kept either at 26°C. or 37°C. Only fresh stools were used and only those from males, to avoid admixture of urine. In all cases either normal salt preparations or plain stool specimens were carried as controls.

Entamoeba "A" was supplied by a ten year old boy who had had neither ipecac nor emetine, but who was taking bismuth in large doses. The stools were liquid without either pus or blood grossly, and with very little mucus. A moderate number of the many active organisms contained red blood cells.

Entamoeba "Y" was obtained from a twenty-two year old negro whose stools were composed almost entirely of blood, pus and mucus. The previous week he had received 5 grains of emetine. Many organisms contained red blood cells.

Entamoeba "H" came from a middle aged man who had received a course of emetine nine months previously. The stools contained blood, pus and mucus, and many of the Entamoebae contained red blood cells.

Entamoeba "W" came from a man who had suffered with an intermittent bloody dysentery for one year. No previous medication. The stool was obtained after a saline purge and contained very little mucus and no blood.

Entamoeba "C. S." was obtained from a case of dysentery with liver abscess before any medication was given. Liquid stools with a small amount of blood and pus. Every drop of the stool was swarming with active organisms, some of which contained red blood cells.

The appearance of Entamoebae being destroyed by emetine applied directly is the same as that noted in the case of a man whose Entamoebae disintegrated spontaneously within thirty minutes after passage, following ipecac by mouth, 50 grains on four consecutive days. The organisms continue in active motion for ten or fifteen minutes, then become quiet and circular. A little later all the granular contents and nucleus would retire to one side and apparently shrink to one-fourth or one-fifth of the total volume of the cell, leaving the bulk of the cell clear and homogeneous, with the refraction normal to the ectosarc. At this time the whole cell seemed to be swelling a little in size.

At times semicircular waves can be seen running from the periphery of the cell to the periphery of the granular mass lying against one side of the cell circumference. In the majority of instances, after ten to fifteen minutes, the granular contents again become diffused throughout the entire cell leaving no clear margin, and the refraction begins to fade. In a few minutes there is a slight movement of the organism as though it were about to turn over, but no ectosarc is protruded. Just after this movement is started the periphery breaks in one place and some of the granular contents nearest the breaking point begin to flow out. At the instant the periphery breaks, the entire cell boundary disappears and the refraction fades entirely, leaving only an oblong spot of granules to represent the corpse. In other instances after the granular mass has rediffused throughout the cell, there is no movement with rupture of the periphery, but a rapid fading of the refraction, the cell retaining its circular outline for hours: these faded *Entamoebae*, without any refraction and without any differentiation into ectosarc and endosarc are dead and resemble degenerating epithelial and pus cells. In strong solutions the protozoa may swell rapidly and burst. In undisturbed preparations of lethal emetine dilutions and *Entamoebae* it is possible to pick out many of the areas of granules which recently have been living organisms.

In trying the effect of emetine solutions on *Paramecia*, as stated above, Rogers found his organisms killed in one hour in 1:200,000 dilutions. Using a similar ciliate, 1:5000 was the weakest dilution the writer found to have any effect within one hour.

Dale and Dobell point out that many substances such as quinine and methyl-psychotrine are more toxic for *Entamoebae* than emetine *in vitro*, and yet have no effect when administered for amoebic dysentery, in comparatively large doses, whereas emetine in very small doses is very effective. They further found that while emetine will cure amoebic dysentery in man, it has no effect on the same *Entamoebae* when transferred to kittens. Hence they suggest, "that the therapeutic efficacy of emetine is a result of its action upon the host rather than upon the parasite."

TABLE 1

SOURCE OF EMETINE	DILUTION	ENT-AMOEBA	STARTING TIME	TIME OF OBSERVATION	EFFECTS NOTED	DATE
Parke, Davis and Company.....	1:15,000	A	4.20	4.50 5.20	None None	December 20
Parke, Davis and Company.....	1:7,500	A	4.48	4.58 5.18 6.00	None None None	December 20
Parke, Davis and Company.....	1:150	A	6.10	6.20	95 per cent dead Control motile	December 20
Merek.....	1:1,875	A	5.05	5.25 5.35	Sluggish Disintegrated Control motile	December 20
Merek.....	1:1,875	A	6.05	6.17 6.45	Sluggish $\frac{2}{3}$ disintegrated Control motile	December 20
Mulford.....	1:150	A	7.00	8.00	None	January 6
Mulford.....	1:75	A	6.45 7.25	7.25 7.50	None Motility good	January 6
Burroughs, Wellcome and Company	1:7,500	A	5.30 6.30	6.10 6.30 7.40	None None Very motile	January 6

Burroughs, Wellcome and Company.....	1:150	A	$\left\{ \begin{array}{l} 6.13 \\ 7.13 \end{array} \right\}$	6.45 7.13 7.35	Very motile Very motile Very motile	January 6
Merek.....	1:1,875	A	$\left\{ \begin{array}{l} 5.20 \\ 6.40 \end{array} \right\}$	6.10 6.40 7.40	None None Motility good	January 6
Merek.....	1:150	A	$\left\{ \begin{array}{l} 6.15 \\ 7.15 \end{array} \right\}$	6.47 7.15 7.42	Motility good Motility good Motility good	January 6
Merek.....	1:75	A	$\left\{ \begin{array}{l} 6.20 \\ 7.20 \end{array} \right\}$	6.42 7.20	Motility good Motility good	January 6
Quinine HCl.....	1:100	A	$\left\{ \begin{array}{l} 7.20 \\ 5.40 \end{array} \right\}$	7.45 8.30	Motility good Motility good	January 6
Parke, Davis and Company.....	1:15,000	Y	$\left\{ \begin{array}{l} 4.30 \\ 5.00 \end{array} \right\}$	1.55 5.20	Unaffected Unaffected	December 20
Parke, Davis and Company.....	1:7,500	Y	$\left\{ \begin{array}{l} 5.00 \\ 5.40 \end{array} \right\}$	5.15 6.00	Unaffected Unaffected	December 20
Parke, Davis and Company.....	1:150	Y	$\left\{ \begin{array}{l} 5.10 \\ 6.00 \end{array} \right\}$	5.55	Majority disintegrated	December 20
Merek.....	1:1,875	Y	$\left\{ \begin{array}{l} 5.10 \\ 6.00 \end{array} \right\}$	5.35 6.00	Sluggish Disintegrated	December 20

TABLE 1—Continued

SOURCE OF EMETINE	DILUTION	ENT-AMOEBA	STARTING TIME	TIME OF OBSERVATION	EFFECTS NOTED	DATE
Merek.....	1:1,875	Y	6.20	7.00 7.20	Sluggish One-half disintegrated One-half sluggish	December 20
Merek.....	1:3,600	Y	3.35	4.17 4.35	Unaffected Unaffected	December 23
Parke, Davis and Company.....	1:2,400	Y	5.05	6.15 6.45	Unaffected Unaffected	December 23
Parke, Davis and Company.....	1:1,200	Y	4.53	5.45 6.20 6.40	Unaffected Unaffected Unaffected	December 23
Parke, Davis and Company.....	1:600	Y	4.47	4.57 5.20	Unaffected Unaffected	December 23
Parke, Davis and Company.....	1:600	Y	5.38	5.48 6.00 6.20 6.40	Unaffected Unaffected Unaffected Unaffected	December 23
Parke, Davis and Company.....	1:300	Y	4.03 4.25*	4.25 4.45 6.00 6.20	80 per cent motile 50 per cent motile 50 per cent quiet, 40 per cent motile and 10 per cent disintegrating Same	December 23

Parke, Davis and Company.....	1:150	Y	{ 3.55	4.00 5.00 6.30	Quiet Degenerating 1 motile amoeba	December 23
Merek.....	1:1,875	Y	{ 1.08 1.30	1.30 2.10 2.30	Unaffected Unaffected Unaffected	December 28
Merek.....	1:75	Y	{ 1.00 1.15 1.35	1.35 2.00 2.30	Unaffected Unaffected Unaffected	December 28
Mulford.....	1:75	Y	2.12	2.30	Unaffected	December 28
Merek.....	1:1,875	II	{ 12.30 1.30	1.30 1.40 2.15 3.30	Unaffected Unaffected Unaffected Unaffected	December 29
Merek.....	1:150	II	4.30	5.30	Unaffected	December 29
Merek.....	1:75	II	{ 1.25 1.55	1.45 1.55 2.05 2.15 3.40	Unaffected Amoebae quiet, dead, or sluggish Amoebae all quiet Amoebae dead 90 per cent disintegrated	December 29
Burroughs, Wellcome and Company....	1:150	II	4.16	5.30	Unaffected	December 29

TABLE 1—*Concluded*

SOURCE OF EMETINE	DILUTION	ENT-AMOEBA	STARTING TIME	TIME OF OBSERVATION	EFFECTS NOTED	DATE
Mulford.....	1:150	W	9.06	9.30 9.55 10.27	Unaffected Unaffected Unaffected	March 11
Mulford.....	1:75	W	10.04	10.40	Very few motile	March 11
Parke, Davis and Company.....	1:7,500	W	8.20	9.00 9.55 10.29	Unaffected Unaffected Unaffected	March 11
Burroughs, Wellcome and Company....	1:150	W	10.00	10.30	Very motile	March 11
Merek.....	1:3,600	W	8.52	9.58	Unaffected	March 11
Merek.....	1:1,875	W	8.25	9.25	Unaffected	March 11
Merek.....	1:150	W	9.10	9.52 10.23	Unaffected Very motile	March 11
Merek.....	1:75	W	10.08	10.48	50 per cent quiet NaCl control motile 2.5 hrs.	March 11
Burroughs, Wellcome and Company....	1:75	C. S.	8.41	10.20	Disintegrated	May 24
Burroughs, Wellcome and Company ..	1:150	C. S.	8.40	10.16	Disintegrated	May 24
Burroughs, Wellcome and Company ..	1:300	C. S.	8.38	10.14	Disintegrated	May 24
Burroughs, Wellcome and Company ..	1:600	C. S.	8.36	10.10	Sluggish	May 24
Burroughs, Wellcome and Company....	1:1,200	C. S.	8.33	10.05	Sluggish	May 24

Burroughs, Wellcome and Company...	1:2,500	C. S.	8.28	10.02	Unaffected	May 24
Burroughs, Wellcome and Company...	1:5,000	C. S.	8.26	10.00	Unaffected	May 24
Burroughs, Wellcome and Company...	1:10,000	C. S.	8.24	9.58	Unaffected	May 24
Mulford.....	1:100	C. S.	8.22	9.56	Few and quiet	May 24
Mulford.....	1:200	C. S.	8.20	9.53	All motile	May 24
Mulford.....	1:400	C. S.	8.19	9.51	All motile	May 24
Mulford.....	1:800	C. S.	8.16	9.47	All motile	May 24
Mulford.....	1:1,600	C. S.	8.15	9.45	Both quiet and motile	May 24
Mulford.....	1:3,200	C. S.	8.12	9.41	amoebae	May 24
Mulford.....	1:6,400	C. S.	8.10	9.40	Unaffected	May 24
Mulford.....	1:12,800	C. S.	8.08	9.38	Unaffected	May 24
Merek.....	1:75	C. S.	8.06	9.33	Disintegrated	May 24
Merek.....	1:150	C. S.	8.04	9.30	All dead	May 24
Merek.....	1:300	C. S.	8.01	9.25	Few disintegrating	May 24
Merek.....	1:600	C. S.	8.00	9.15	Sluggish but motile	May 24
Merek.....	1:1,200	C. S.	7.58	9.10	Sluggish but all motile	May 24
Merek.....	1:2,500	C. S.	7.55	9.08	Unaffected	May 24
Merek.....	1:5,000	C. S.	7.50	9.06	Unaffected	May 24
Merek.....	1:10,000	C. S.	6.55	7.25	Quiet	May 24
				8.45	Motility good	May 24
				10.20	Motility good	May 24
Tap water.....		C. S.	7.30	9.50	Motility good	May 24

* Coverglass removed and a new supply of emetine thoroughly stirred into the feces, scattering the organisms singly over the field.

TABLE 2

REAGENT	DILUTION	MIXING TIME	TIME OF OBSERVA- TION	EFFECTS NOTED, OCTOBER 23, 1914
Chrysoidin.....	1:2,000	4	4.00	Motion ceased and disintegration started at once
	1:8,000	4.07	4.27	Motion sluggish and circular
			4.45	Motionless
	1:8,000	12.11	12.26	Motion sluggish
			12.40	Only circular motion
	1:2,000	4.15	4.15	Motion ceased and swelling and disintegration started at once
Anilin grün.....	1:8,000	4.23	4.23	Motion ceased at once
	1:16,000	4.34	4.50	Very sluggish
			5.04	Motionless
	1:32,000	4.29	4.51	Very sluggish
			5.09	90 per cent motionless; 10 per cent revolving
	1:150	12.00	12.05	Sluggish
Emetine HCl (Burroughs, Wellcome and Company)	1:1,200	12.11	12.08	Locomotion gone; revolving
			12.59	Swelling
			12.31	Swelling
			1.00	Disintegrating rapidly
			12.20	Motion slight
			12.30	Motionless
	1:2,400	1.07	12.59	Swelling
			1.15	Disintegrating
	1:4,800	12.23	1.37	A few active; majority sluggish
			2.11	Many swelling. None motile
			12.33	Motion good
			12.58	Motion good
Quinine HCl.....	1:200	12.03	1.35	Motion somewhat lessened but still good
			12.03	Motion ceased at once
			12.10	Ovoid
			12.29	Refraction gone, form retained
	1:3,200	12.08	1.04	Disintegrating rapidly
			12.12	Sluggish
			12.26	Motionless: majority disintegrated
			1.02	95 per cent disintegrated
	1:6,400	12.15	12.25	Motion good
			1.01	Motion sluggish
			1.15	Only revolving motion

Equal parts of the reagent and a fresh 0.15 per cent broth culture of a free-living ciliate (*Paramecium* or *Colpidium*) were mixed on slides at room temperature.

Macht and Fisher (13), noting the close chemical relationship between emetine and the papaverin alkaloids of opium, find all these alkaloids toxic for protozoa in vitro, and show that the toxicity "is due to the benzyl grouping present in their molecules." It should be instructive to see what action benzyl benzoate proves to have on *Entamoebae* in man. Macht (14) reports one case and Haughwout, Lantin and Asuzano (15) report ten cases of acute amoebic dysentery treated with benzyl benzoate. In all the motility of the bowel was controlled, the blood and pain disappearing rapidly, with prompt clinical recovery. In six the last stool examined was amoeba-free, but as none of these cases could be followed after clinical recovery, we are still without any reliable data concerning the effect of this drug on *Entamoeba* in man.

SUMMARY

Using emetine and *Entamoeba histolytica* from various sources, in no instance did emetine affect the appearance or motility of these organisms in the stools in dilutions weaker than 1:2000 in the space of one to two hours, and often dilutions as strong as 1:150 failed to kill the *Entamoebae* in the allotted time.

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THE CLOTTING EFFICIENCY OF THROMBOPLASTIC AGENTS: A REPLY.

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In a recent issue (1) of this Journal F. Fenger of Armour and Company has taken us to task about the variable results on clotting activity obtained with one specimen of Thromboplastin (Armour) on different beef plasmas. The criticism is limited in scope but the conclusions are so sweeping and misleading that I have asked the editor of the Journal to grant me brief space in which I may emphasize essential points which have been undoubtedly overlooked by Fenger in his general charge of carelessness, neglect and unfairness.

The results of our work have been previously published (2) in sufficient detail and are available to anyone who is interested. Restated, our aim was “. . . to compare the thromboplastic activity of several different products; the activity of fresh and old preparations. . . .” This was accomplished by the application of the method of Howell and that described in N. N. R., testing several different products on the same plasma, using kephalin as the standard and saline as control. In other words all conditions of the test were the same except the product. Such a procedure necessarily gives an idea of the comparative value of the different products. Therefore, no matter how variable the plasmas the results obtained must give an idea as to the comparative value of the products. In other words, the leading object of our study was not the method of testing, but the comparative behavior of different preparations.

In this respect, it is obvious that Fenger misleads himself. He cites the variable results of several of our experiments with

Thromboplastin (Armour) on different plasmas, admitting the variable response of oxalate plasmas by his own method of testing. Fenger does not mention the results with other thromboplastic agents used in these same experiments. Without these it would be obviously impossible to obtain an idea of the thromboplastic activity of Thromboplastin (Armour) as compared with other agents. As a matter of fact the results obtained with Thromboplastin (Armour) when compared with different agents on the same plasma and on different plasmas agreed in the same direction and were quite favorable. In other words the results from different experiments made at different times were concordant. This satisfies completely "the most important criterion of any test or method of analysis," about which Fenger interrogates us as follows, "Does the method of procedure give concordant results, not only when duplicate tests are made simultaneously, but also when the same test is repeated at different times?"

Now as to the variability in the clotting time of different plasmas with Thromboplastin (Armour), the item of our work which Fenger urges so heavily against us. Variability, of course, is perfectly obvious in the results of table 2 of our paper not only with Thromboplastin (Armour), but also with all the other agents tested. This is as would be expected with any biological reaction known to me, even the clotting time of blood and plasma. All of our results were recorded as obtained under the conditions set forth in our paper. No results were eliminated. No selection was practiced. Incidentally, I know of no publications or data by Fenger either as to the variability in response of different plasmas or as to results with different thromboplastic agents. None are presented in his criticism, although he freely admits that variability in plasmas exists. Concerning his method of selection or preparation of a suitable plasma, he leaves us entirely in doubt. This is highly regrettable. The publication of all results, whether positive or negative, favorable or unfavorable, is very desirable indeed. This should remove such misunderstanding and suspicion illustrated by Fenger's criticism.

CONCLUSIONS

It is obvious that Fenger's criticism of the variability of results with Thromboplastin (Armour) in our work on the clotting activity of various thromboplastic agents is not pertinent.

The methods of testing used by us, that of Howell, and a modification of this by Fenger (N. N. R.), are admittedly simple of application, and, so far as these methods are reliable at all, our technique was reliable. The data and results reported by us are correct, and we can not follow Fenger's advice to accept only certain results. On the contrary it is necessary to record and report all results, and such was the case in our work. Under these conditions it is obvious that our conclusions were as fair as the analysis of the results warranted. Until Fenger publishes results or presents some evidence concerning the comparative value of different thromboplastic agents, inclusive of the methods of testing these, his criticism of our work must remain valueless and unfounded.

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THE CARMINATIVE ACTION OF VOLATILE OILS¹

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Volatile oils, or substances containing them, have been used as medicinal agents from the earliest times. The therapeutic aims have been numerous, but perhaps the most frequent is to produce the "carminative" effect.

The term "carminative" has been employed for at least five centuries. It is derived either from "carmen" a "song" or "charm," or from "carminare" to "card wool," suggesting that they diluted or carded out the humours that give rise to flatulence. Their action is to diminish feelings of distension and discomfort in the stomach, usually with eructations of gas, and to allay or prevent griping in the intestine. This gastro-intestinal effect has led to the popular use of most of the group of volatile oils at one time or another, and has been the ground for their presence as correctives in many compound pharmacopoeal preparations used as purgatives.

Apart from the fact that in many instances they certainly do act "like a charm," very little is known as to the nature of the action, the relief of pain and distension being purely subjective.

EXPERIMENTAL

The action of volatile oils on the isolated intestine

The movements of the isolated intestine were recorded by the method of Magnus. Pieces of intestine of the rabbit, rat and cat were suspended in oxygenated Locke's or Tyrode's solution.

¹ The experiments on which this paper is based were performed in the Pharmacology Department of University College, London, during 1913 and 1914. Military service has prevented the publication of the paper till now.

kept at a temperature of 38°C. in an apparatus similar to that described by Dale and Laidlaw (1). Volatile oils were shaken up with Locke's solution to form an emulsion. Measured quantities of this, heated to 38°C., were added to the bath containing the intestine and the effects produced recorded. The bath contained 200 cc. so the final concentration of oil in the bath could be easily determined.

The action of the following volatile oils was investigated: Camphor, oils of anise, carraway, cassia, cinnamon, cloves, fennel, peppermint, rosemary and turpentine. All had the same action on the intestine, decreasing the rate and extent of the

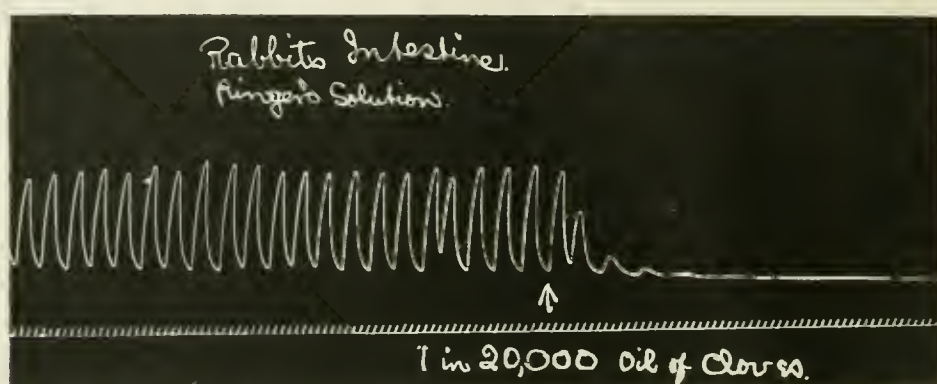


FIG. 1. REGULAR PENDULUM MOVEMENTS OF THE ISOLATED SMALL INTESTINE OF THE RABBIT

Oil of cloves (1 in 20,000) was added at the arrow and the movements quickly stopped.

movement, and causing relaxation. This action was produced with concentrations of 1 in 20,000 and upwards. No increase of movement was seen with smaller concentration in any of the experiments. The action was reversible, for the movement was completely restored when the solution was replaced with fresh Locke's or Tyrode's solution.

With suitably arranged doses an antagonism can be demonstrated between volatile oils and such stimulants of intestinal movement as acetyl-choline, barium chloride, pilocarpine and physostigmine. If the volatile oil were added to the bath and inhibition produced, large doses of those substances were required

to produce their ordinary effect; if they were added first and stimulation produced, this quickly gave place to relaxation on addition of the oil. The action of the oils seems to be directly on the muscle.

Figure 1 shows the typical effect of volatile oils on the isolated intestine, the regular pendulum movements being quickly stopped by 1 in 20,000 oil of cloves.

Action of volatile oils on the intestine in situ

These experiments on the surviving intestine were controlled by a series of twenty experiments in which the movements of the intestine in situ were recorded, by the following method.

The animal (rabbit or cat) was anaesthetised with urethane and ether, and a cannula inserted into the trachea. It was then immersed in a bath of Ringer's solution and the abdomen opened. The two limbs of a Cushny myocardiograph were attached to the small intestine at points about $1\frac{1}{2}$ inches apart. About 2 inches above the proximal lever the point of a very fine glass cannula was inserted and held in place by a purse-string suture of fine silk. To the cannula was fastened about 3 inches of fine flexible rubber tubing. The tubing and cannula together held 0.5 cc. The movements of the intestine were recorded in the usual way. After a definite amount of Ringer's solution was injected into the intestine through the cannula, as a control, the same amount of an emulsion of volatile oil in Ringer's solution was injected, and the effects compared.

The experiments on the intestine in situ for the most part confirmed the observations on isolated intestine. In fourteen experiments there was a distinct relaxation produced, in some cases this amounted to complete inhibition such as is shown in figure 3. In the remainder, the injection of volatile oils in Ringer's solution did not differ from the injection of the same amount of Ringer's solution.

Figure 2 shows the movements of the rabbit's intestine as recorded by the myocardiograph. The apparent increase in tone after injecting Ringer's solution is mechanical, due to the slight

distension shortening the distance between the limbs of the myocardiograph and thus giving the appearance of increased tone.

Figure 3 shows the subsequent injection of 0.5 cc. of 0.05 per cent oil of coriander, causing relaxation and inhibition of movement.

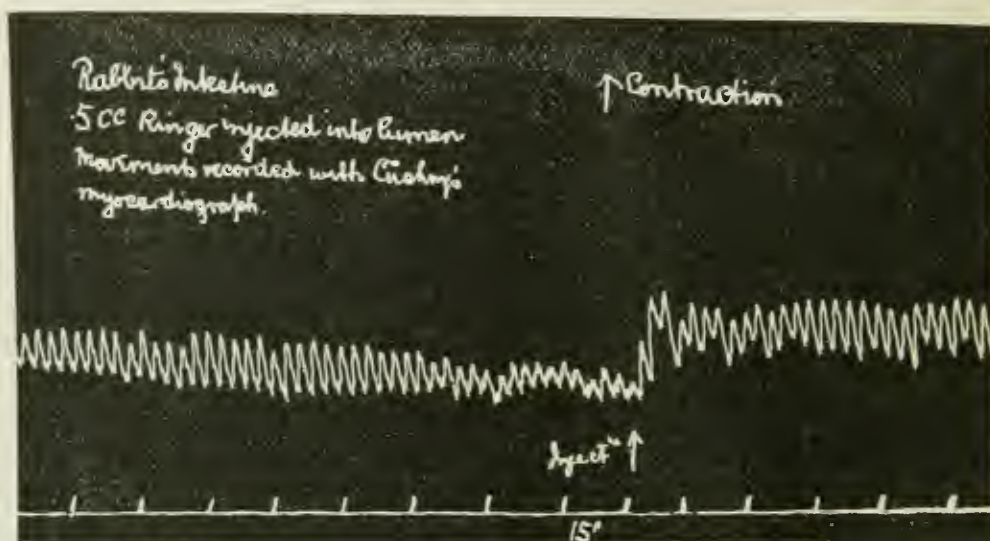


FIG. 2. THE MOVEMENTS OF THE RABBIT'S INTESTINE IN SITU

Recorded with Cushny's myocardiograph. At the arrow, 0.5 cc. Ringer's solution was injected into the lumen.

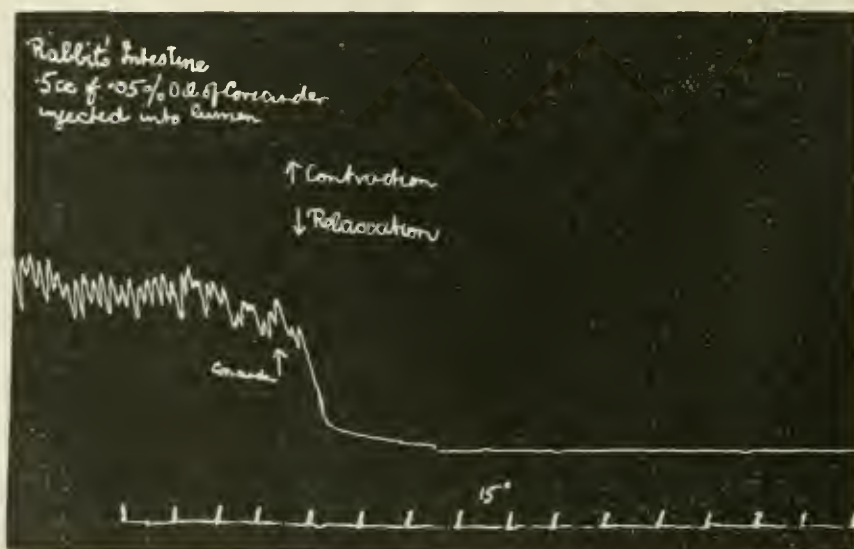


FIG. 3. SAME EXPERIMENT AS FIGURE 2

At the arrow 0.5 cc. of 0.05 per cent oil of coriander in Ringer arrests movement

Action of volatile oils on the excised stomach

Rings of various portions of the stomach of the cat and rabbit, and the whole excised stomach of the rat were suspended in the same way as the intestine. With concentrations of 1 in 20,000 of oils a diminution of movement was produced. Volatile oils had a similar action on the cardiac and pyloric sphincters, and on the oesophagus.

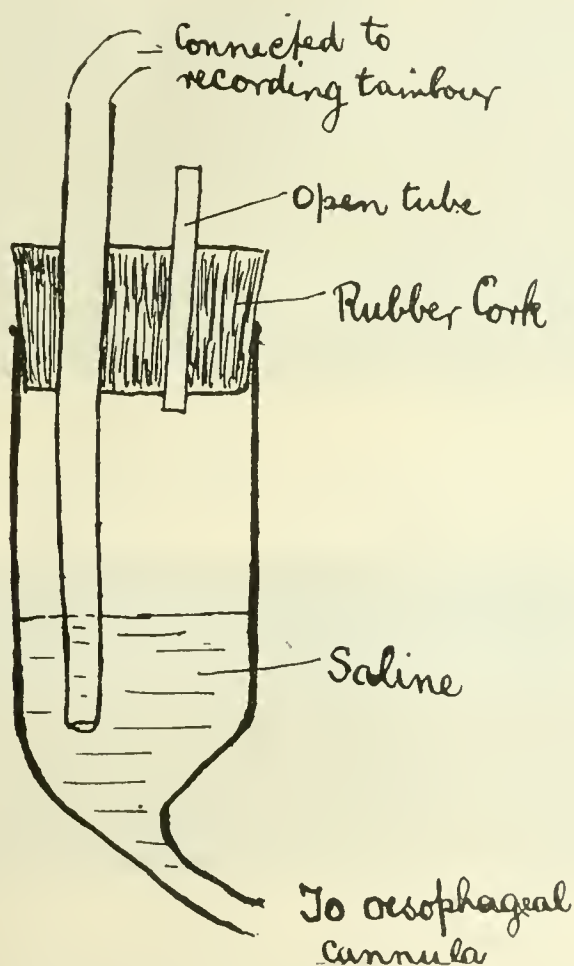


FIG. 4. PRESSURE BOTTLE FOR STOMACH

Action of volatile oils on the stomach in situ

The movements of the stomach of the cat and rabbit were recorded in the following way. The animals were anaesthetised with urethane and ether, some were afterwards decerebrated and pithed. A cannula was inserted into the trachea, and the animal placed in a bath of warm Ringer's solution. The abdomen

was opened and the pylorus tied. A cannula was passed along the oesophagus into the stomach, and tied at the cardiac orifice. The stomach was partly filled with warm saline, and the oesophageal cannula attached to a pressure bottle having a cross-section of $2\frac{1}{2}$ inches, so that a comparatively large movement of

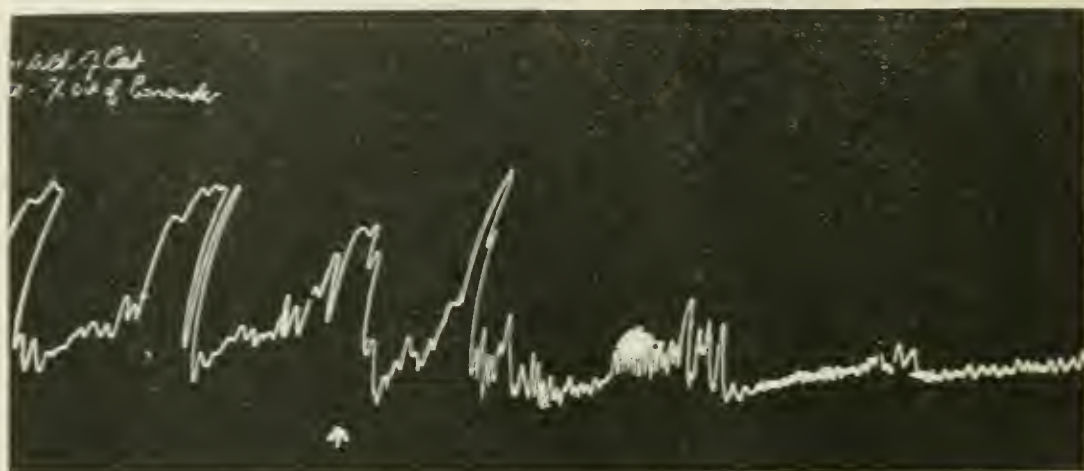


FIG. 5. MOVEMENTS OF STOMACH OF CAT IN SITU
Injection of 2 cc. of 5 per cent oil of coriander into stomach

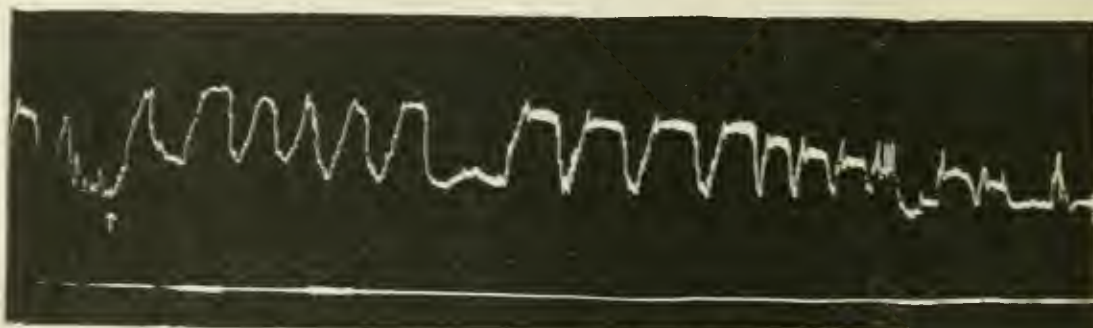


FIG. 6. MOVEMENTS OF STOMACH OF CAT IN SITU
Injection of 2 cc. 0.5 per cent oil of coriander

fluid into or out of the stomach did not raise the level much. The bottle was attached to a recording tambour, and raised a few cms. until the maximum amount of movement was obtained. The arrangement is shown in figure 4. The emulsions of oil in Ringer's solution were injected into the stomach through a fine hypodermic needle. Twenty-two experiments were performed,

with the following results. In twelve there was diminution of movement; in six small doses of oil produced no effect; and in four there was a distinct increase in movements. Increase of movement was noted after oil of mustard. In three out of four experiments on pithed cats the decrease in movement was marked; in the fourth there was no effect.

Figure 5 shows an experiment in which injection of 2 cc. of 0.5 per cent oil of coriander quickly produced inhibition of movement; figure 6, one in which the same amount of oil had but little effect, the movements decreasing to half their extent after some time.

The action of volatile oils on other unstriated muscle

Macht (2), von Kurdinowski (3) and Prochnow (4) have shown that volatile oils inhibit the uterine movements. The mammalian heart, if perfused through the coronary arteries with 1 in 30,000 oil of coriander, is slowed after one minute, systole is less complete, and the rate of flow through the coronary arteries is quickened by 50 per cent. It has been shown by J. A. Gunn (5) that oil of turpentine dilates mammalian blood-vessels.

Since my experiments were completed Muirhead and Gerald (6) have stated that in pieces of surviving suspended intestine some of the oils in dilutions of 1 to 50,000 cause increased tone and sometimes some increase in the pendulum movements, while in greater concentration (1 to 10,000) they relax the muscle and arrest movement; others appear to cause relaxation even at the lower concentration. Salant and Mitchell (10) found that oil of chenopodium decreased tone and slowed or arrested contractions in dilutions of 1 to 10,000; in the carnivora this phase of depression was usually preceded by one of increased tone. The intravenous injection of the oil was followed by lessened peristalsis in the rabbit.

I did not observe the stage of increased activity in any of my experiments on isolated tissues. The first effect was always diminished tone and movement, which progressed to complete paralysis when the concentration was increased. In my experi-

ments the high dilutions used by Muirhead and Gerald were not employed, and their results for stronger concentrations agree with mine and those of Salant and Mitchell. The augmentor action in all cases seems to be slight and transient and the characteristic feature of the action of the volatile oils is diminished tone and contraction.

DISCUSSION OF RESULTS

If a few minims of volatile oil be taken by mouth, eructation of gas follows in a few minutes. It seems probable that this is due to dilatation of the cardiac sphincter and consequent passage of the gas into the lower end of the oesophagus. The oil is usually administered in the form of a spirit, after food, when the stomach is full. The oil, being so slightly soluble, will take some time to be distributed through the stomach, and will act strongly on the cardiac end and cause dilatation. If the eructations be explained, as is sometimes done, by saying that carminatives increase the movements of the stomach, it is difficult to explain why the gas passes backwards into the oesophagus, and is not driven on into the pylorus and duodenum.

After some time the oil becomes mixed with the food in the stomach, and there is a condition comparable to the experiments on the stomach *in situ*, in which the oil in solution or emulsion was injected into the stomach, containing saline, through which the oil would probably be distributed immediately on injection. It seems possible that the effect of volatile oils on the movements of the stomach depends on the relative strength of two actions, a direct action on the muscle causing relaxation, and a reflex, arising from irritation of the mucous membrane of the stomach, causing contraction. The other actions of volatile oils on the oesophagus and stomach, namely, the sense of warmth and well-being is probably due to the slight local irritation.

For the action on the intestine the preparations usually given are those in which there is some colloid material to prevent absorption, such as cinnamon and ginger. The experimental findings afford a good explanation of their action in preventing grip-

ing, by stopping excessive movements of the intestine. In the experiments on the intestine in situ this action was not invariably produced, but, on the other hand, in man prevention of griping by carminatives is not invariable.

Volatile oils are stated by Brandl (7), Scanzoni (8), and Farnsteiner (9), to cause accelerated absorption from the stomach and intestines. Part of their action may be due to this factor.

Oil of turpentine is frequently added to enemata in the proportion of an ounce to a pint of enema. When this is administered in tympanitis due to parietic conditions of the bowel it is usually followed by passage of large quantities of flatus. The action is probably a reflex increase of peristalsis following irritation of the rectal mucous membrane, for the direct action of oil of turpentine of this concentration is to cause immediate paralysis of unstriated muscle. It is possible that the depressant action may aid this effect by relaxing some irregular contractions which hinder the passage of the gas through the bowel.

SUMMARY

1. The carminative action of volatile oils can best be explained by their relaxing and inhibiting the movements of plain muscle.
2. Their other effects on the gastro-intestinal tract may be explained by their local irritant action, by reflexes arising therefrom, or possibly by acceleration of absorption.

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THE INFLUENCE OF REACTION ON THE PRECIPITATION OF PROTEINS BY TANNIN

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INTRODUCTION

The effects of typical astringents depend upon the precipitation of protein. In the case of tannins, the precipitation is greatly influenced by the reaction (H-ion concentration). This is particularly important when tannin drugs are administered internally, to produce local effects on the alimentary canal, with its varying reactions.

The data in the literature are scanty and rather confusing, since they were gathered for the most part before the distinction between actual reaction and titrable reaction was clearly realized. The only investigation which takes this properly into account was that of Hanzlik (1915); and as this was made for another purpose, it dealt with dilutions that cannot be safely transferred to the ordinary conditions of astringent action.

Summary of qualitative statements as to reaction on tannin-protein precipitation. The following seems to be a fair review of the statements of the literature.

Proteins, including albumen, glue, blood, peptones and pepsin, are precipitated by tannin, in neutral or faintly alkaline, or very feebly acid media only; these precipitates being redissolved by the addition of acids (even acetic) or strong alkalies (hydroxids, carbonates, borax), but not by weaker alkalies (sodium bicarbonate or disodium phosphate). They are also soluble in excess of protein.

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Discordant statements are made as to "alkali-tannate." This is, tannin neutralized by sodium bicarbonate; and is probably of inconstant composition and therefore of inconstant reaction. According to some, the watery solutions do not precipitate proteins, but are nevertheless astringent to tissues. (Heinz, 1889; Lewin, 1904.) This only means that they become precipitant as soon as their excess of alkali has been absorbed by the tissues, and when, in other words, they have reached the H-ion concentration of the tissues.

Similarly, Gebhardt, 1899, found that all alkaline tannin solutions precipitate proteins when used as a "ring" test; i.e., at the point in the test-tube where the suitable H-ion concentration is reached.

The re-solution of the tannins in excess of protein is presumably due to a similar cause; i.e., an unsuitable reaction of albumin or blood; although further work is desirable.

Rost, 1897, believed that precipitation of protein occurs only in faintly acid reaction. Dixon conceived the precipitation as due to the acid radical (H-ion concentration?) of the tannin. Both statements would imply that tannin could not precipitate in the presence of a neutral or feebly alkaline buffer solution.

Incidentally, tannin precipitates also non-protein colloids, for instance, starch.

Direct determination of H-ion concentration on tannin-protein precipitation. The only data that have been published are those of Hanzlik, 1915, in his investigation of the relation of alkaloidal precipitants to the isoelectric point of serum. This is the reaction at which the positive and negative charges held by the protein ions are about equal, and where there is a maximum of neutral particles of proteins (Michaelis). It lies about pH 4.74. The precipitation by ethyl alcohol reaches its maximum at this point.

Hanzlik used horse-serum and glutin, dialyzed for five to six weeks, i.e., practically salt free. The experiments were made by adding one drop of 1 per cent tannin, and one drop of 0.05 per cent serum or 0.5 per cent glutin to 2 cc. of buffer-mixture. The final mixture therefore contained about 0.017 per cent serum and 0.02 per cent of tannin.

With these conditions, precipitation appeared with pH 4.14; reached its maximum at pH 4.74; and was absent with pH 5.35.

The maximum precipitation therefore occurred at the isoelectric point, and at a distinctly acid reaction. Other "alkaloidal precipitants" behaved differently, but do not concern us in this connection.

Increase of the serum content to 1 per cent shifted the optimal reaction slightly toward the alkaline side.

Neutral salts, such as chlorid or sulphocyanid had little effect of the precipitation.

OBJECTS OF PRESENT INVESTIGATION

The deficiencies and contradictions of the literature indicated the desirability of determining anew the optimum and threshold H-ion concentrations, using higher concentrations of tannin than those employed by Hanzlik. Krameria and catechu were also tried, as representing the "natural vegetable astringents." Gallic acid was tested, as our samples were somewhat precipitant to protein under suitable conditions.

Serum-protein, egg-albumen and "peptone" (Witte) were used, as representative of the proteins that the tannin would encounter in the tissues or contents of the alimentary canal.

GENERAL METHOD OF EXPERIMENTATION

This consisted in mixing the tannin, and protein in the presence of a buffer solution of the desired reaction, adding the indicator, and then sufficient acid or alkali to secure the desired change in the indicator. It was aimed to cover a range between pH = 1 to 10.

Three series of experiments were made, differing in the quantity of buffer added. They will be given in chronologic order, although experiment 1 contained the least buffer, and it therefore the least accurate.

The percentage of the chemicals in the finished mixtures was as follows:

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
Serum, dried.....	1	$\frac{1}{4}$	$\frac{1}{10}$
Albumen, moist.....	5	$2\frac{1}{2}$	1
Peptone.....	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{10}$
Tannin.....	$\frac{1}{2}$	$\frac{1}{4}$ and $\frac{1}{16}$	$\frac{1}{3}$
Krameria.....	$1\frac{1}{2}$ (extract)	$1\frac{1}{4}$ (root)	
Catechu.....	$1\frac{1}{4}$		
Gallic acid.....	$\frac{3}{8}$	$\frac{3}{8}$	

Original protein solutions. These were made up in 0.9 per cent NaCl on the preceding afternoon and filtered during the night.

S = *Serum*; dried serum, 1 per cent, dissolved by trituration with the solvent, standing half an hour, and filtering through paper.

A = *Albumen*; 10 per cent of fresh egg-white, squeezed with the solvent through cheese cloth, and filtered.

P = *Peptone*; Witte's dry: 1 per cent warmed with the solvent and filtered.

Designation of effects: These are ordered as:

$$\begin{array}{lcl} - & = & \text{no change} \\ T & = \text{turbidity} & \left\{ \begin{array}{l} \text{sl} = \text{slight} \\ \text{st} = \text{strong} \end{array} \right. \\ + & = \text{precipitate} & \left\{ \begin{array}{l} \text{sl} = \text{slight} \\ \text{st} = \text{strong} \end{array} \right. \end{array}$$

Method in experiment 1. The tannin was dissolved in 0.9 per cent sodium chlorid solution, with the addition of sodium bicarbonate, of the percentage stated under each experiment. This was intended to act as a buffer.

Ten cubic centimeters of these solutions were mixed with 0.5 cc. of indicator, the required amount of acid or alkali, and sufficient 0.9 per cent NaCl to make 20 cc. These solutions therefore contained one-half of the original quantity of tannin, 5 cc. of the solutions were then mixed with 5 cc. of the protein solutions. The final mixtures therefore contained one-fourth of the original tannin and one-half of the original proteins.

The buffer value is small, and the reaction is further liable to change by the final addition of the protein solutions.

Method in experiment 2. In this, the reagents were mixed in the presence of appropriate buffer solutions, and the pH adjusted after the addition of the protein.

The following solutions were used.

Protein solutions. The same as in experiment 1.

Protein buffer solutions. These were made by adding 1 volume of protein solution to 2 volumes of the buffer solutions, made up according to Sørensen-Michaelis (glycocoll; citrate; phosphate; borax). The indicators were added and if the tint differed from that of the original buffer-solution, $\frac{N}{10}$ HCl or 1 per cent sodium carbonate were added, until the tints matched.

Astringent solutions. The following were used; the solvent in all being 0.9 per cent sodium chloride.

Tannin, 1 per cent; sodium bicarbonate, $\frac{1}{2}$ per cent

Tannin, $\frac{1}{4}$ per cent; sodium bicarbonate, $\frac{1}{4}$ per cent

Krameria extract, 5 per cent; sodium bicarbonate, $\frac{1}{2}$ per cent

Gallic acid, $1\frac{1}{2}$ per cent; sodium bicarbonate, $\frac{2}{3}$ per cent

Order of mixture. Five cubic centimeters of the astringent solution were added to 15 cc. of the buffer-protein solutions.

Method of experiment 3. This differed from experiment 2 only in having a lower ratio of protein to tannin, in a higher proportion of buffer.

The buffer-protein mixture was made with 10 cc. of buffer solution and 1 cc. of protein solution. In the test, 5 cc. of tannin solution were added to the mixture.

SUMMARY OF RESULTS

These are presented as graphs, in figures 1 and 2; the depth of the shading corresponding to the density of the precipitation. The symbols are those already explained. "S" stands for serum; "A" for albumen (egg white) and "P" for Witte peptone.

The charts show that the relation of the tannin-protein precipitation to hydrogen-ion concentration varies somewhat with conditions; but is broadly similar. The outstanding features are as follows:

1. There is practically no difference in the behavior of serum, albumen, and peptone.

2. The maximum of precipitation is between pH 2 and 5.

3. The extremes of reaction that permit precipitation by tannin lie between pH = less than 1 and pH = 8 to 8.3. The acid threshold varies with the concentration of tannin. With $\frac{1}{2}$ per cent of tannin, the precipitation at pH = 1 is still dense. With $\frac{1}{3}$ to $\frac{1}{4}$ per cent, there is moderate precipitation or strong turbidity at this reaction. With $\frac{1}{16}$ per cent of tannin, there is merely turbidity beyond pH = 2.

4. Variation of the protein content of two and one-half times (experiments 2 and 3) affected only the amount of precipitation, but not the relation of the reaction.

P_H	Tannin 1%			Tannin 1%			Tannin 1/2%			Krameria			Gallic			P_H
	S	A	P	S	A	P	S	A	P	S	A	P	S	A	P	
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	9
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10

FIG. 1. EFFECT OF HYDROGEN ION CONCENTRATION ON TANNIN PRECIPITATION

Experiments 2 and 3. S = serum; A = albumen; P = peptone. Experiment 3 contained a lower ratio of protein. The shading indicates the intensity of precipitation.

P_H	Tannin			Krameria			Catechu			Gallic Ac.			P_H
	S	A	P	S	A	P	S	A	P	S	A	P	
1	+	+	+	+	+	+	+	+	+	+	+	+	1
2	+	+	+	+	+	+	+	+	+	+	+	+	2
3	+	+	+	+	+	+	+	+	+	+	+	+	3
4	+	+	+	+	+	+	+	+	+	+	+	+	4
5	+	+	+	+	+	+	+	+	+	+	+	+	5
6	+	+	+	+	+	+	+	+	+	+	+	+	6
7	+	+	+	+	+	+	+	+	+	+	+	+	7
8	+	+	+	+	+	+	+	+	+	+	+	+	8
9	+	+	+	+	+	+	+	+	+	+	+	+	9
10	+	+	+	+	+	+	+	+	+	+	+	+	10

FIG. 2. EFFECT OF HYDROGEN ION CONCENTRATION ON TANNIN PRECIPITATION

Experiment 1. This is less reliable than figure 1, since it contained less buffer. Nevertheless, it shows a general agreement. The range of the tannin is greater because the concentration of the tannin was higher than in curve 1.

5. *Krameria* and catechu behave essentially like dilute tannin. The precipitation limits are the same.

6. Gallic acid has the same precipitation maximum as tannin; but the extremes lie between $\text{pH} = 1$ to 2 and $\text{pH} = 5$ to 6. Presumably, the precipitation was due to the admixture of a trace of tannin.

Comparison with results of Hanzlik. Hanzlik, working with $\frac{1}{50}$ per cent tannin, found the maximum of precipitation at $\text{pH} = 4.74$, and the extremes between 4.14 and 6.35. The differences conform with the tendency of dilution of tannin in my experiments; i.e., the shifting of the maximum toward the alkaline side, and the approach of the extremes in both directions.

APPLICATION OF THE RESULTS TO THE DIGESTIVE TRACT

The results of these experiments render materially clearer the understanding of the possible action of tannins in the digestive tract.

Mouth. The pH of saliva is quoted by Michaelis as 6.9. At this reaction, tannin, *krameria* and catechu are distinctly precipitant. This agrees with the marked astringency of these precipitants in the mouth.

Stomach. The resting empty stomach may be nearly neutral. During the first one and one-half to three hours after a meal, the acidity rises rapidly to $\text{pH} = 2$ or even 1; depending on the individual. It then remains stationary until nearly all the food has left the stomach (McClendon, 1915).

The reaction of the stomach comes well within the limits suitable for the tannin-precipitation.

If tannin is taken without food, it would exert considerable astringent action on the neutral or slightly acid stomach. If it is taken with food there would also be some astringency in the gastric mucosa; but the precipitation would concern mainly the proteins of the food. The precipitation would increase at first with the increasing acidity of the gastric contents; but before the end of the gastric digestion is reached, a part of the precipitate would be redissolved; not because of the formation of "peptones"

for these are equally precipitable, but because of the excessively acid reaction.

The chyme as it passes the pylorus would therefore almost always contain tannin in three forms: as tannin-protein, precipitate; free tannin adsorbed into the precipitate; and tannin-protein held in solution by the acidity of the chyme. The adsorption is doubtless an important factor; for it is difficult to remove the excess tannin from tannin protein precipitates by simple washings.

Duodenum. The reaction of the duodenal contents is the resultant of the neutralization of the acid chyme by the slightly alkaline intestinal and pancreatic juice and bile. The approximate pH of these is stated as: intestinal juice, 8 (Michaelis); pancreatic juice, 8.3 (Michaelis); bile 7.8 (Okada). The extreme alkalinity would therefore be about 8 to 8.3, i.e., just about the limit of tannin-precipitation.

The actual pH of the duodenal contents, however, is much more acid. Myers and McClendon, 1920, found the usual reaction in man to be between 3.2 and 5, when the precipitation would be optimal.

As the occurrence of astringent effects depends on whether the reaction in the duodenum is more or less favorable to precipitation than that of the gastric chyme, it is evident that astringency would occur whilst the over-acid chyme was in process of neutralization; but that it would probably cease when the acidity falls below $\text{pH} = 7$.

Small intestine. Long and Fenger, 1917, found the reaction of the intestinal contents slightly acid throughout the small intestine; the acidity, decreasing somewhat with the distance from the pylorus. In the human jejunum, it ranged from $\text{pH} = 3.8$ to 7.8, mean 5.8; in the upper third of the small intestine in hogs, it ranged from 6.6. to 7.4, mean 6.7; in the middle third, 6.5 to 7.6, mean 6.9; in the lower third, 6.4 to 8.1, mean 7.

Similar figures were obtained in calves, lambs and dogs. They have been confirmed by McClendon et al., 1920.

These reactions fall well within the precipitation limits of tannin with protein. However, the reaction becomes progressively less favorable from the duodenum onward. There would

consequently be no astringency beyond the duodenum, except in proportion as new supplies of tannin become available by the complete cleavage of the proteins. This would liberate the tannin that had been bound or adsorbed by the protein. However, this tannin would be promptly fixed by the cells of the intestines. Astringent action could therefore extend only to that level of the intestines at which the cleavage of protein is practically completed.

This ordinarily would be long before the ileo-cecal valve is reached. Accordingly, it would be quite impossible to obtain astringent effects in the large intestine, unless peristalsis or protein cleavage are very abnormal, as for instance in cholera. This agrees with the observation that normally, the feces do not show any tannin after the administration of large doses (8 grams) of the drug. (Moerner, 1892.) The conditions with "insoluble" tannin are different, as will be discussed in another paper.

CONCLUSIONS

1. The precipitation of proteins by tannin depends on the hydrogen-ion concentration of the medium. With concentrations of tannin such as would be used for astringent effects ($\frac{1}{10}$ to $\frac{1}{2}$ per cent), precipitation is maximal between $\text{pH} = 2$ to 5 ; with greater acidity or alkalinity, the precipitation becomes smaller. With alkalinity greater than $\text{pH} = 8$ or 8.3 there is no precipitation, and therefore no astringency. The limits are somewhat more narrow for dilute than for concentration solutions of tannin.

2. The precipitation limits are essentially the same for native proteins (serum and albumen) and for "Witte peptone." They are therefore not materially affected by digestion unless this goes beyond the stage of albumoses and probably peptones.

3. *Krameria* and catechu have the same limits as ordinary tannin. Gallic acid produces only very slight precipitation, within the same limits as tannin, and probably due to contamination with this.

4. These data suggest the following as to astringency by tannic acid in the alimentary tract:

a. The resting empty stomach would permit precipitation of the mucosa, and therefore astringency or irritation of the stomach. The tannin would thus be bound probably completely and prevented from acting on the intestine.

b. When taken with food there would be some astringent action but the precipitation would involve mainly the food proteins. These precipitates would also adsorb some of the tannin. The precipitation would at first increase with the acidity; but at the maximum acidity, a part would be redissolved.

c. The chyme, as it reached the duodenum, would contain tannin in the form of protein precipitates, adsorbed tannin, and redissolved tannin.

d. In the duodenum, the conditions would be favorable to further precipitation, and therefore to astringent action.

e. The reaction of the entire small intestines would permit precipitation, if any tannin were available. However, the reaction becomes progressively less favorable from the duodenum downward. Consequently, no further precipitation could occur after the duodenum or jejunum, except by the liberation of bound tannin. Such liberation occurs to some extent by the ultimate cleavage of the protein by which the tannin had been bound or adsorbed. However, this cannot extend only so far in the intestine as protein-cleavage extends; for the liberated tannin would be immediately bound by the intestinal mucosa.

It is therefore impossible that the astringent action should extend to the large intestines, unless peristalsis or protein cleavage are very abnormal.

g. Solutions of krameria or catechu would behave like tannin, and it is difficult to see how they could have much advantage. The crude drugs or solid extracts might possibly be superior, namely, if they dissolve only slowly in the intestines.

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THE STABILITY OF BENZYL ALCOHOL SOLUTIONS

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INTRODUCTION

Next to the efficiency of anesthesia and the safe limits of toxicity, the two requirements which are essential for the practical clinical use of local anesthetics are ease of sterilization and satisfactory keeping quality of their solutions. In a paper published by one of the authors (M.) some two years ago in this Journal, the discovery of the anesthetic properties of benzyl alcohol was described and it was shown, both experimentally and clinically, that solutions of this substance give excellent anesthesia when infiltrated into tissues, and that this anesthetic is by far the least toxic of all the common local anesthetics in use (1). An inquiry into how well this drug satisfied the above two requirements was therefore very desirable. Solutions of benzyl alcohol can be kept indefinitely in a sterile condition for two very good reasons. In the first place, the high boiling point of this anesthetic (204.7°C.) permits its solutions to be sterilized by boiling. In the second place, through the investigations of Macht and Nelson (2), and Macht, Satani and Swartz (3), it was shown that even weak solutions of this drug are markedly germicidal. In the present communication, the authors wish to report some observations on the stability or keeping properties of benzyl alcohol.

METHOD

Specimens of solutions of benzyl alcohol of different concentrations (1 to 4 per cent) were prepared by the authors from chemically pure benzyl alcohol and kept in sealed ampoules for

different periods of time, varying from a few days to two years and longer. Other specimens of benzyl alcohol solutions were obtained on the market, and also examined after various intervals of time.

The anesthetic efficiency of the various solutions was studied most conveniently on the cornea of the rabbit's eye, and, in some cases also, on the cornea of the dog's eye, in the usual fashion, which need not be described in this place. Especial attention was paid to the time of onset of the anesthesia, to its duration, and to the local irritation, if any was present.

The efficiency of these preparations was tested from time to time. To our surprise, we found that certain samples had lost a great part of their ability to produce anesthesia. A search for the cause of this led to the correlation between the hydrogen ion concentration and the stability of the sample.

The hydrogen ion concentration measurements were made by the colorimetric method, using the standard solutions and indicators developed by Clark and Lubs (1917) (4). These were checked by the electrometric method. The indicators have the property of changing color in narrow zones of hydrogen ion concentration. In this case the variations were so great that two indicators giving a more alkaline range than Clark and Lubs indicators were employed. These were obtained from Sørensen's indicators. The indicators used were:

Thymol sulphon phthalein.....	pH 1.3- 2.8
Tetrabrom phenol sulphon phthalein.	pH 2.8- 4.6
Methyl red.....	pH 4.6- 5.6
Dibrom cresol sulphon phthalein.....	pH 5.6- 6.8
Phenol sulphon phthalein.....	pH 6.8- 8.4
Phenol phthalein.....	pH 8.4-10.0
Alizarin yellow R.....	pH 10.1-12.1
Tropaeolin O.....	pH 11.1-12.7

As a means of orientation, it may be stated that pH 7.0 represents true neutrality, that pH 2.0 is 0.01 NaHCl and pH 12.0 is 0.01 N NaOH. A recent review of the significance and determination of hydrogen ion concentration will be found in the article by Shohl (1920) (5).

EFFECT OF DIFFERENT KINDS OF GLASS

When a solution of pure benzyl alcohol is made in water or in physiological saline, the hydrogen-ion concentration or "reaction" of such a solution is practically neutral. Even when such solutions are made with hot water or saline, in order to hasten the solubility of the drug, the hydrogen-ion concentration remains very nearly neutral (pH 7.0–6.8). When such solutions, however, are kept standing for long periods of time, certain chemical changes take place, as evidenced by the hydrogen-ion concentration, which bear a direct relation to the kind of glass in which the solutions are kept. The following two protocols will serve as striking illustrations.

Experiment 1. A 4 per cent solution of pure benzyl alcohol, prepared by the author (M.) in the spring of 1918, which was kept in a nonsoluble glass measuring flask, exposed to the light; at the ordinary room temperature, was tested for its anesthetic efficiency, at various intervals of time, giving the following results:

April 17, 1918, complete anesthesia of rabbit's cornea in 1 minute; duration of anesthesia over 30 minutes; no irritation; pH = 6.8.

June 7, 1920, complete anesthesia of rabbit's cornea in 1 minute; duration of anesthesia over 30 minutes; very slight reddening of conjunctive; pH = 5.2.

Experiment 2. An ampoule of 4 per cent benzyl alcohol solution was obtained on the market. The ampoule, as subsequent inquiry revealed, was made of ordinary soft glass. Repeated tests with this solution gave the following results:

June 10, 1918, complete anesthesia of rabbit's cornea in 1 to 1½ minutes; duration of anesthesia 30 minutes; no irritation; pH = 6.8.

December 10, 1918, complete anesthesia of rabbit's cornea in 2 to 2½ minutes; duration of anesthesia 5 minutes; slight reddening of the conjunctive.

May 26, 1920, very little anesthesia of rabbit's cornea produced even after prolonged application of the drug; distinct reddening of the conjunctiva; solution looks slightly turbid; pH = 8.0.

In view of the marked difference in the keeping qualities of the benzyl alcohol solutions, as shown by the above two protocols, a more careful study of the effects of glass on the solutions was un-

dertaken. Four lots of pure benzyl alcohol were obtained from two different manufacturers. Solutions of 1 to 4 per cent of these in water were made and then sealed in three kinds of ampoules. The initial hydrogen-ion concentration of all the solutions varied from pH 7.0 to pH 6.8, and their anesthetic efficiency was initially the same in all cases. The solutions were put up in ampoules of (1) flint, or soft glass, (2) soft amber-colored glass, and (3) non-soluble or hard glass. These ampoules were opened from time to time and the anesthetic efficiency was tested on the cornea of the rabbit or dog. The results obtained were extremely interesting and are exhibited in the following protocols:

Experiments with lot A

I. Ampoules of flint glass

April 17, 1919, complete anesthesia of cornea in 1 minute; no irritation; duration of anesthesia, 20 minutes.

December 11, 1919, complete anesthesia of cornea in 2 minutes; slight irritation; duration of anesthesia, 15 minutes.

June 4, 1920, complete anesthesia of cornea in $2\frac{1}{2}$ to 3 minutes; reddening of conjunctiva; duration of anesthesia 10 minutes.

pH of original solution 6.8; pH on June 4, 1920, 8.0.

II. Ampoules of amber glass

April 17, 1919, complete anesthesia of cornea in 1 minute; no irritation; duration of anesthesia 20 minutes.

December 11, 1919, complete anesthesia of cornea in 2 minutes; slight irritation of conjunctiva; duration of anesthesia 15 minutes.

June 4, 1920, complete anesthesia in 3 minutes; reddening of the conjunctiva; duration of anesthesia about 5 minutes.

pH of original solution 6.8; pH on June 4, 1920, 12.0.

III. Ampoules of nonsoluble glass

April 17, 1919, complete anesthesia in 1 minute; no irritation; duration of anesthesia 20 minutes.

December 11, 1919, complete anesthesia in 1 minute; no irritation; duration of anesthesia 20 minutes.

June 4, 1920, complete anesthesia in 1 minute; no irritation; duration of anesthesia over 30 minutes.

pH of original solution 6.8; pH on June 4, 1920, 4.2.

Experiments with lot B

I. Ampoules of flint glass

April 20, 1919, complete anesthesia of cornea in 1 minute; duration of anesthesia, 20 minutes; no irritation.

June 4, 1920, complete anesthesia in $2\frac{1}{2}$ to 3 minutes; duration of anesthesia 10 minutes; slight irritation of conjunctiva.

pH of the original solution was 6.8.

pH on June 4, 1920 was 12.0.

II. Ampoules of amber glass

April 20, 1919, complete anesthesia of cornea in 1 minute; duration of anesthesia 20 minutes; no irritation.

June 4, 1920, complete anesthesia in 4 minutes; duration of anesthesia 5 minutes; reddening of the conjunctiva.

pH of the original solution was 6.8.

pH on June 4, 1920 was 12.0.

III. Ampoules of non-soluble glass

April 20, 1919, complete anesthesia of cornea in 1 minute; duration of anesthesia 20 minutes; no irritation.

June 4, 1920, complete anesthesia of cornea in 1 minute; duration of anesthesia 30 minutes; no irritation.

pH of original solution was 6.8.

pH on June 4, 1920 was 4.2.

Experiments with lot C

Ampoules of nonsoluble glass

In this series a 4 per cent solution of benzyl alcohol, put up in hard glass ampoules, was obtained on the market, and gave the following results:

March 15, 1919, complete anesthesia of the cornea in 1 minute; duration of anesthesia 20 minutes; no irritation.

May 26, 1920, complete anesthesia of cornea in 1 to $1\frac{1}{2}$ minutes; duration of anesthesia 20 minutes; no irritation.

The pH of the original solution was not tested, but the pH on May 26 was 5.0.

Experiments with lot D

Ampoules of nonsoluble glass

A 2 per cent solution of benzyl alcohol was put up in hard glass ampoules and repeated examinations of the same gave the following results:

March 19, 1919, complete anesthesia of rabbit's cornea in $1\frac{1}{2}$ minutes; anesthesia of dog's cornea in 2 minutes.

April 8, 1919, complete anesthesia of rabbit's cornea in 1 minute; of dog's cornea in 2 minutes; no irritation.

May 23, 1919, complete anesthesia of rabbit's cornea in $1\frac{1}{2}$ minutes; of dog's cornea in 2 minutes; no irritation.

June 3, 1920, complete anesthesia of rabbit's cornea in 1 minute; of dog's cornea in 2 minutes; duration of anesthesia in each case about 15 minutes.

pH of original solution was 6.8.

pH on June 4, 1920 was 4.5.

Thus, it is readily seen that in those solutions where there was a loss of anesthetic power, there was in every case an alkaline reaction. This alkaline reaction developed only in the flint and amber colored glass. The solutions in the non-soluble and pyrex glass remained neutral or acid and retained their anesthetic properties.

EFFECT OF BOILING WITH ACID AND ALKALI

In order to study further the relation between the hydrogen-ion concentration and the anesthetic efficiency of benzyl alcohol solutions, the authors investigated the effect on the drug produced by adding small amounts of acid or alkali. For this purpose a 3 per cent solution of benzyl alcohol was taken and 10 cc. of it were introduced into each of three hard-glass ampoules. To the solution in one of the ampoules a buffer reagent (acid and

alkaline phosphate) was added, so as to render the hydrogen-ion concentration equal to pH 6.8. To the second ampoule 1 cc. of a tenth normal solution of hydrochloric acid was added, rendering the reaction equivalent to pH 2.0. To the third ampoule, 1 cc. of a tenth normal solution of sodium hydroxide was added, rendering the reaction equivalent to pH 12.0. The ampoules were then carefully sealed and boiled in a water bath for two hours. On the following day the ampoules were opened and the anesthetic action of each solution was tested on rabbits and dogs. It was found that the acid solution of benzyl alcohol produced complete anesthesia of the rabbit's cornea in one to two minutes and that the duration of the anesthesia lasted fully twenty minutes. The anesthetic efficiency of the neutral solution was practically the same as of the preceding one. On the other hand, the alkaline solution of benzyl alcohol did not produce complete anesthesia of the rabbit's cornea until the application lasted for three to four minutes, and the duration of the anesthesia was not longer than five minutes. Exactly parallel results were obtained on testing the anesthetics on the dog's cornea. There was no doubt, whatever, as to the impaired efficiency of the benzyl alcohol solution to which alkali had been added.

DISCUSSION

It will be seen from the experiments above described that the activity of benzyl alcohol solutions is markedly affected by the nature of the glass in which they are contained, the soft or alkaline glass tending to impair its efficiency rather rapidly, while the hard or non-alkaline glass did not do so. The reaction of a freshly prepared solution of pure benzyl alcohol is pH 7.0 to 6.8, or practically neutral. Such a solution, when kept in a non-soluble glass container, remains unchanged in its reaction for quite a long time. Its hydrogen-ion concentration may increase slightly on standing for many months to pH 5.0, or even 4.2. Such an increase in the hydrogen-ion concentration does not in any way impair its anesthetic efficiency, nor is the acidity of such solutions great enough to produce any irritation in infiltration

anesthesia of general surgical work, though possibly such solutions may produce a little primary irritation of the conjunctiva. It should be noted, however, that many samples of benzyl alcohol obtainable on the market contain excessive amounts of benzaldehyde; and inasmuch as benzaldehyde quickly oxidizes to benzoic acid, on the one hand, while pure benzyl alcohol undergoes such oxidation very slowly, in preparing solutions of the latter drug for clinical use, care should always be taken to remove all benzaldehyde from the benzyl alcohol before dissolving it in water. This can easily be done by treating with sodium bisulphite and subsequent washing of the drug.

Of much greater importance for practical purposes is the effect of alkalies on solutions of benzyl alcohol. As the authors have shown, benzyl alcohol solutions kept in soft-glass containers rapidly deteriorate. The alkali of the glass hastens the oxidation of the alcohol to benzoic acid and indeed is sufficient not only to neutralize such acid as may be formed, but actually to render the solution alkaline in reaction. Such solutions were found to have lost in a great measure their anesthetic efficacy. Such a deterioration in the potency of a drug produced by alkaline glass is not entirely unknown. A similar impairment in the potency of strophanthin solutions, traceable to the effects of soft glass, has been reported recently by Levy and Cullen (6).

In the present case, differing from the case just cited, it is possible to suggest a mechanism of this deterioration. Here the chemical reaction of oxidation indicated by the equation:
$$2 \text{C}_6\text{H}_5\text{CH}_2\text{OH} + \text{O}_2 \rightarrow 2 \text{C}_6\text{H}_5\text{CHO} + 2\text{H}_2\text{O} + \text{O}_2 \rightarrow 2 \text{C}_6\text{H}_5\text{COOH}$$
is hastened by neutralizing the benzoic acid formed and thus allowing the oxidation to progress more rapidly and to greater completion; thus leaving less benzyl alcohol present in solution.

In view of the above changes produced by the various forms of glass, it is evident that the best method of preserving benzyl alcohol solutions is to prepare such solutions from benzaldehyde-free alcohol and to seal the same in containers made of hard glass, with the addition of a small amount of buffer solution which would keep the hydrogen ion concentration unchanged at about pH 7.0 to 6.8.

SUMMARY

1. Various specimens of benzyl alcohol solutions were sealed in ampoules made of different kinds of glass, and such solutions were examined at intervals of time in regard to their anesthetic efficiency on the one hand, and their hydrogen-ion concentration on the other.

2. Solutions of benzyl alcohol kept in non-soluble glass preserve their anesthetic properties completely for long periods of time and such solutions tend to increase their hydrogen-ion concentration very slowly.

3. Benzyl alcohol solutions kept in soft glass or alkaline containers tend to become alkaline in reaction and rapidly deteriorate in their anesthetic efficiency.

4. It is suggested that the best method of preserving benzyl alcohol solutions is to prepare such solutions from a benzyl alcohol free from benzaldehyde, and to seal the same after the addition of a buffer solution in hard glass ampoules.

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ERRATA, VOL. XVI, NO. 2, SEPTEMBER

Page 71. Line 23 from bottom, for "secretory" read "salivary."

Page 72, before "slower" insert "generally;" after "rabbit" insert "when blood is collected in this way."

Page 74, line 14 from bottom, for "find" read "found;" line 13 from bottom, for "incredibly" read "extraordinarily."

Page 107, line 4, delete "Whatever action;" line 12, for "in" read "with."

THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS VI. ATROPINE; PILOCARPINE

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The well-known peripheral action of atropine and of pilocarpine on structures innervated by parasympathetic nerves, especially the heart and secretory glands, and on the secretion of sweat, which is under the influence of true sympathetic nerves, suggests that these drugs might exert an action on the secretion of epinephrin from the adrenal glands. There is, at present, no well established information bearing on this question, based upon experimental evidence obtained with methods that are sufficiently sensitive and capable of yielding quantitative results.

Meltzer and Auer (1) observed that the enucleated frog's bulbus reacted to small amounts of adrenalin by dilatation and suggested that this might prove to be a better reagent than the blood-pressure to demonstrate the efficiency of a suprarenal preparation. Ehrmann (2), employing this reaction, concluded that in rabbits and cats there is no noteworthy effect produced by atropine or pilocarpine on the epinephrin secretion from the adrenals. He obtained blood through a cannula in the cava just below the entrance of the renal veins. The renal vessels were tied at the hilus, the abdominal aorta and small veins entering the cava above the cannula were tied and a ligature on the cava just beneath the liver completed a pouch which permitted only the blood from the adrenal and first lumbar veins to flow through the cannula. Serum was obtained from this blood by centrifuging and diluted five, ten and twenty times with physiological salt solution, and applied to the frog bulbus. Systemic blood-serum, in like dilutions, was used as a control. Ehrmann states that the concentration

of adrenalin in the adrenal vein blood of the rabbit is between 1:1,000,000 and 1:10,000,000 and in the cat it is considerably less, but he does not take account of the differences in the rate of blood-flow through the adrenals. The greater concentration of epinephrin in the adrenal blood of the rabbit than in that of the cat can be explained by the slower flow through the adrenals in the rabbit. Differences in the amount of dilution of the adrenal vein blood by the blood from the first lumbar veins would also contribute to the differences in the epinephrin concentrations in the bloods collected by Ehrmann. His experiments yield no quantitative information on the rate at which epinephrin was being secreted from the adrenals.

The enucleated frog's eye as a test for epinephrin was carefully studied and critically considered by Schultz (3). He found that the degree of mydriasis is not proportional to the strength of the adrenalin solution applied. At 23°C. solutions which contain less than 1:625,000 adrenalin yield a reaction so small as to be of uncertain origin. Schultz also makes the pertinent suggestion that substances in sera, other than adrenalin may materially modify the reaction. A test object that cannot be relied upon to detect, with certainty, concentrations of epinephrin below 1:625,000 can, at best, be of use only for qualitative reactions. Such a concentration of epinephrin, under ordinary experimental conditions, is very nearly the maximal found in adrenal vein blood collected with the slowest blood-flows, in the absence of splanchnic stimulation, massage of the glands or administration of certain drugs (nicotine), which are capable of augmenting the output of epinephrin to yield a concentration above the ordinary possible maximum.

Popielski (4) studied the effect, on the blood-pressure, of releasing the aorta after clamping it off in the thorax for three to seven minutes. Although he made no quantitative observations, he found no alteration in the degree of the reaction after administering atropine. The epinephrin factor in the blood-pressure reaction thus obtained is contributed by the entrance into the circulation of the epinephrin which is secreted into the adrenal capillaries and veins during the period of occlusion of the aorta and is washed into the blood stream when the circulation through the glands is re-established. We have found, in similar experiments, that short periods of anemia, thus produced, do not materially interfere with the secretion of epinephrin from the adrenals, and that, after release of the aorta, the epinephrin secreted under the influence of splanchnic stimulation while the circulation through the adrenals was interfered with, is washed into the circulation

and produces the characteristic reactions on the pupil and nictitating membrane of the eye previously rendered sensitive to epinephrin by removal of the superior cervical ganglion (5).

Biedl (6) found no diminution in the epinephrin secretion from the adrenals, after administration of atropine, on stimulation of the splanchnic nerve. Tchekoksaroff (7), employing the same method as Biedl, confirmed this observation and, further observed that no demonstrable effect on the epinephrin secretion was produced by administration of pilocarpine. His experiments were performed on dogs. The abdomen was opened in the midline from the xiphoid to the symphysis and a transverse incision then made on the left side of the abdomen parallel with and near the costal margin and the flaps laid open. The abdominal viscera, covered with warm towels, were laid over to the right side, a cannula inserted into the left lumbar vein and the left adrenal vein tied at its entrance into the cava, permitting only the blood coming from the left adrenal to flow through the cannula. The rate of blood-flow through the adrenal, at the time of collection of the blood, was taken account of by counting the number of drops that flowed from the cannula in a given time. The blood, after defibrination, was then injected, in 10 cc. quantities, into the jugular vein of another dog weighing 5 to 8 kgm., and the effect on the blood-pressure and heart action observed. Tchekoksaroff found that adrenal vein blood collected during stimulation of the peripheral end of the splanchnic nerve, after administration of 10 mgm. of atropine, produced fully as great an effect on the blood-pressure and heart action, when introduced into another dog, as the blood collected before atropine was injected. Pilocarpine, administered in doses of 5 to 10 mgm, after section of the splanchnic, produced a copious flow of saliva and pancreatic juice, but the adrenal vein blood collected after the injection of pilocarpine caused no greater effect, when introduced into the circulation of another dog than the blood collected before the drug was injected, or than the introduction of an equal quantity of arterial blood. In the experiments performed by Tchekoksaroff the splanchnic nerve was sectioned and only the peripheral effects of these drugs could have been observed. The interference with the nerve supply to the adrenal in some of his experiments apparently had so diminished the epinephrin output from the adrenal that the blood from the gland gave no greater reaction than arterial blood. The method of assay of the adrenal vein blood, employed by him, although not so sensitive as the use of segments of rabbit's intestine and uterus, is capable of yielding quantitative data.

Dale and Laidlaw (8) investigated the action of pilocarpine on the rate of output of epinephrin from the adrenals in cats. They employed, as a test object for epinephrin, the isolated non-pregnant cat's uterus and collected blood from the adrenals through a cannula in a pouch of the cava. In one experiment they used a loop of rabbit's intestine as the test object. The animal was pithed, the abdomen opened and the whole alimentary canal, from the cardiac orifice of the stomach to the lower part of the rectum was removed, after ligation of all the vessels. The portion of cava including both adrenal veins was isolated and a cannula inserted in the lower end for collection of the adrenal vein blood. Fifty milligrams of hirudin was injected into the jugular vein of the cat. In some of the experiments small tributary veins were left unligatured and the adrenal vein blood was diluted considerably. They performed five experiments, in only one of which was there an assay made of the amount of epinephrin in the adrenal vein blood. In experiments 1, 3 and 4 quoted by Dale and Laidlaw they found that pilocarpine had doubled the output of epinephrin per unit of time. Experiment 5 gave the same result although the uterus was not sensitive, while experiment 2 showed no change. In the fourth experiment the concentration of epinephrin in the adrenal blood collected after injection of pilocarpine (blood flow 5 cc. in 83 seconds) was assayed at 1:200,000, giving an output of 0.01 mgm. in 34 seconds. This corresponds to about twenty times the average normal output usually found by us in cats under urethane or ether. Dale and Laidlaw did not assay the epinephrin content of the adrenal vein blood collected before injection of pilocarpine in this experiment, but since they find qualitatively that the output after pilocarpine was doubled, the initial output must have been incredibly high, i.e., about ten times the rate found by us using rabbit's intestine and uterus segments for the assays.

The observations made by Dale and Laidlaw on the eye, after removal of the superior cervical ganglion, could not have yielded quantitative information on the rate of output of epinephrin from the adrenals. Obviously, even qualitative observations on this test object must be cautiously interpreted when studying the effect of a drug like pilocarpine on the adrenal secretion.

Our experiments on the influence of atropine and of pilocarpine on the rate of output of epinephrin from the adrenals were made on cats. The method of obtaining adrenal vein blood has been sufficiently described in previous papers (9). The adrenal speci-

mens were assayed on rabbit's intestine and uterus segments. In all of these experiments, except one with pilocarpine (cat 466), the drugs were introduced through a cannula in the external jugular vein. The actual dose administered would, in every case, be somewhat greater than that mentioned, as the abdominal aorta was tied off near the bifurcation when the cava pocket was made. In general, it may be stated that the results of our experiments lead us to the same conclusions arrived at by other investigators. No significant alteration in the rate of output of epinephrin from the adrenals is caused by either of these drugs. Certainly, any effect that might be demonstrated (if any genuine effect exists) is not comparable to the pronounced influence on the epinephrin output demonstrated by the action of strychnine (9), nicotine (10), and curara (11). Condensed protocols of some of the experiments and samples of tracings used in the assays are given.

THE INFLUENCE OF ATROPINE ON THE EPINEPHRIN OUTPUT

The smallest dose of atropine employed was 0.15 mgm. per kilogram of body weight. This amount always produced marked dilation of the pupils and paralysis of the vagus endings in the heart, but no diminution in the rate of output of epinephrin from the adrenals. Indeed, the assays indicate that there may have been a small augmentation of the epinephrin output. Tchekosaroff suggested that splanchnic stimulation, after administration of atropine, seemed to produce a greater output of epinephrin than stimulation before atropine was injected.

Condensed protocol. Cat 459; female; weight 2.92 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.50 a.m. Cava pocket completed;¹ collected adrenal blood.

¹ In all of the experiments the "cava pocket" was made by tying the renal arteries and veins at the hilus (another ligature was tied around each renal vein just before it enters the cava and the lumbar veins tied just before they cross the adrenals), the abdominal aorta just above the bifurcation, and the small tributaries entering the segment of cava which constitutes the pocket. The cannula was inserted into the lower end of the segment of cava and the pocket completed, just before beginning of collection of a specimen, by placing a clip on the cava just below the liver.

- 9.50 $\frac{1}{2}$ a.m. First specimen, 0.7 gram in 30 seconds (1.4 grams per minute).
- 9.51 a.m. Second specimen, 3.6 grams in 3 minutes (1.2 grams per minute).
- 10.04 $\frac{1}{2}$ a.m. End of intravenous injection of 0.45 mgm. atropine sulphate; blood pressure 90 mm. of mercury.
- 10.05 a.m. Third specimen, 2.1 grams in 1 minute.
- 10.06 a.m. Fourth specimen, 4.35 grams in 2 $\frac{1}{2}$ minutes (1.74 grams per minute); blood pressure 92 mm. of mercury.
- 10.20 a.m. Stimulation of peripheral end of vagus caused no effect on heart rate or blood pressure.
- 10.33 $\frac{1}{2}$ a.m. Fifth specimen, 0.6 gram in 30 seconds (1.2 grams per minute).
- 10.34 a.m. Sixth specimen, 3.45 grams in 4 minutes (0.86 gram per minute); blood pressure 70 mm. of mercury at beginning of collection, falling to 62 mm. of mercury at end.

Obtained another specimen of indifferent blood. Combined weight of adrenals 0.302 gram.

The assay on rabbit's intestine segments showed that the second adrenal specimen, collected before injection of atropine, was somewhat stronger than 1:2,000,000 adrenalin, much weaker than 1:900,000, stronger than 1:2,660,000 (fig. 1, observations 14 and 16) and weaker than 1:1,330,000 (fig. 1, observation 18). It was finally assayed at 1:1,600,000, corresponding to an output of 0.00075 mgm. per minute for the cat, or 0.00026 mgm. per kilogram of body weight. Three other sets of observations corroborated this assay.

The third specimen, collected immediately after injection of atropine, and the fourth specimen, collected 1 $\frac{1}{2}$ minutes after injection of atropine, had the same epinephrin concentration, much greater than 1:2,660,000, weaker than 1:800,000 and very well matched by 1:1,330,000 (fig. 2, observations 26 to 30). After confirmation by other observations, they were taken at 1:1,330,000, corresponding to an output, in the third specimen, of 0.0015 mgm. per minute for the cat, or 0.0005 mgm. per kilogram, and in the fourth specimen, 0.0013 mgm. per minute for the cat, or 0.00045 mgm. per kilogram of body weight.

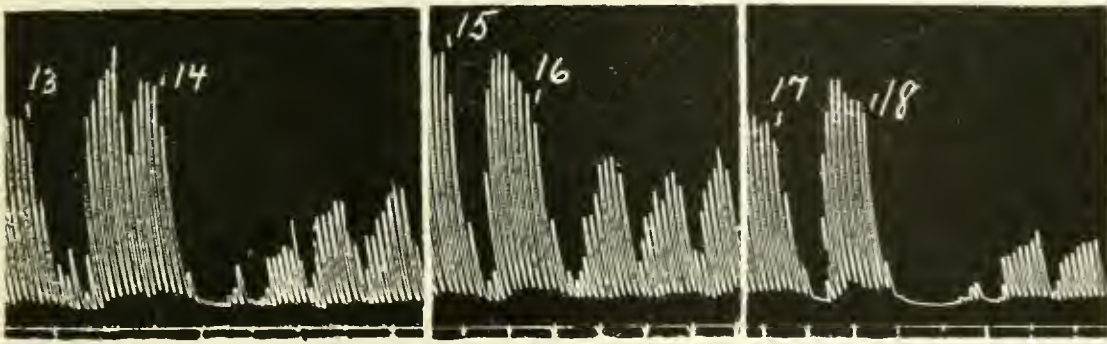


FIG. 1. INTESTINE TRACINGS. BLOOD FROM CAT 459

At 13, 15 and 17, Ringer was replaced by jugular blood and this at 14 by the second adrenal specimen (collected before intravenous injection of atropine) diluted with 4 volumes of jugular blood; at 16 by jugular blood to which was added adrenalin to make a concentration of 1:13,300,000; at 18 by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000. All the bloods were diluted with 5 volumes Ringer (the adrenal blood after dilution with jugular blood and the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

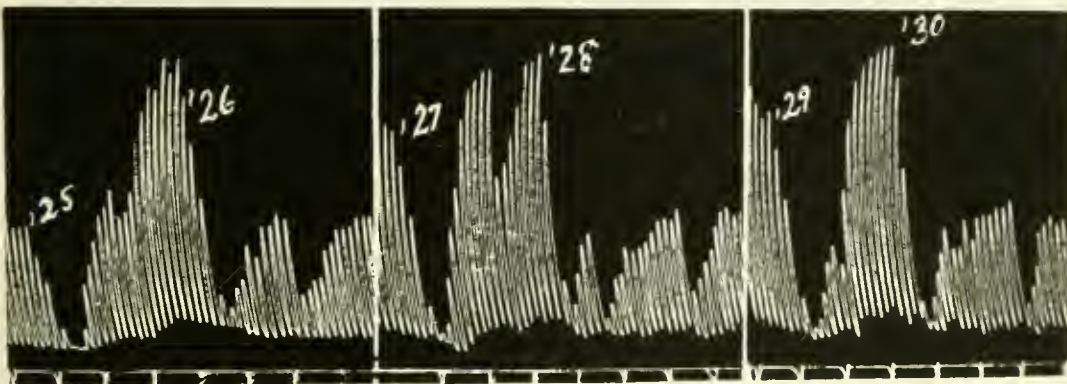


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT 459

At 25, 27, and 29, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 26 by the fourth adrenal specimen (collected 1½ minutes after intravenous injection of atropine) diluted with 4 volumes of indifferent blood; at 28 by indifferent blood to which was added adrenalin to make a concentration of 1:6,650,000; at 30 by the third adrenal specimen (collected immediately after injection of atropine) diluted with 4 volumes indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin and the adrenal bloods after dilution with the indifferent blood). Reduced to two-thirds.

The sixth specimen, obtained 30 minutes after injection of atropine, was weaker than 1:530,000 (fig. 3, observations 40 and 42) and stronger than 1:800,000 (fig. 3, observation 44). This was confirmed on two other sets of observations. It was assayed at 1:700,000, corresponding to an output of 0.0012 mgm. per minute for the cat, or 0.00041 mgm. per kilogram of body weight.

In the next experiment (cat 462) the initial output of epinephrin from the adrenals, before injection of atropine, was low. The animal was pregnant.

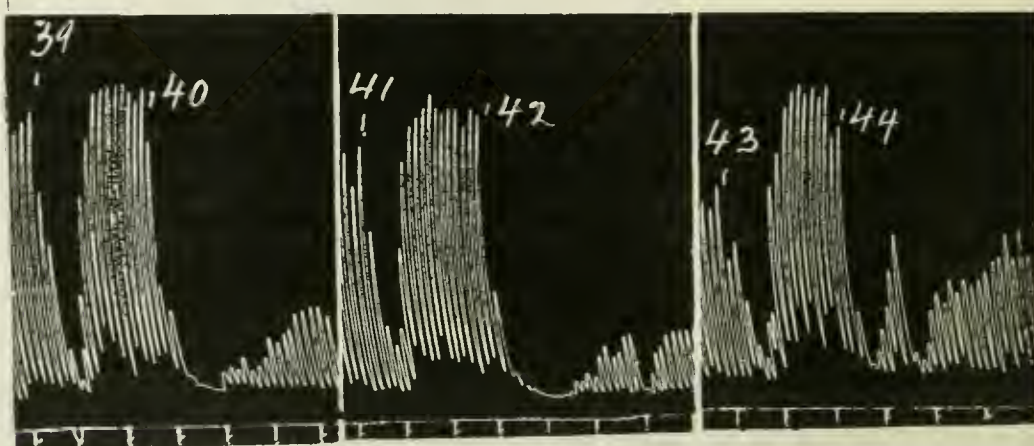


FIG. 3. INTESTINE TRACINGS. BLOODS FROM CAT 459

At 39, 41 and 43, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 40 by the sixth adrenal specimen (collected 30 minutes after injection of atropine) diluted with 4 volumes indifferent blood; at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at 44 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenal blood after dilution with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to three-fourths.

Condensed protocol. Cat 462; female (pregnant); weight 2.55 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.45 a.m. Cava pocket completed; collected adrenal blood.

9.50 a.m. First specimen, 1.1 grams in 30 seconds (2.2 grams per minute).

9.50½ a.m. Second specimen, 3.65 grams in 2 minutes (1.8 grams per minute); blood pressure 82 mm. of mercury.

- 10.01 a.m. End of intravenous injection of 0.4 mgm. atropine sulphate; blood pressure 91 mm. of mercury.
- 10.01½ a.m. Third specimen, 1 gram in 30 seconds (2 grams per minute).
- 10.02 a.m. Fourth specimen, 3.85 grams in 2½ minutes (1.54 grams per minute); blood pressure 74 mm. of mercury.
- 10.12 a.m. Stimulation of peripheral end of vagus caused no effect on heart rate or blood pressure (61 mm. of mercury).
- 10.20½ a.m. Fifth specimen, 0.75 gram in 30 seconds (1.5 grams per minute).
- 10.21 a.m. Sixth specimen, 3.4 grams in 3 minutes (1.13 grams per minute); blood pressure 56 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.310 gram.

The second specimen, obtained before injection of atropine, was decidedly weaker than 1:4,300,000 adrenalin, somewhat weaker than 1:8,600,000, much stronger than 1:14,000,000, and somewhat stronger than 1:10,700,000. It was finally assayed at 1:9,000,000, corresponding to an output of 0.0002 mgm. per minute for the cat, or 0.00008 mgm. per kilogram. The fourth specimen, collected 1½ minutes after injection of atropine, was decidedly stronger than 1:7,000,000, and weaker than 1:2,850,000. It was assayed at 1:4,300,000, corresponding to an output of 0.00034 mgm. per minute for the cat, or 0.00013 mgm. per kilogram. The sixth specimen, obtained 20 minutes after administration of atropine, was decidedly stronger than 1:7,000,000 adrenaline, somewhat stronger than 1:4,300,000, and weaker than 1:2,850,000. It was finally assayed at 1:3,500,000, corresponding to an output of 0.0003 mgm. per minute for the cat, or 0.00012 mgm. per kilogram.

In the above experiments (cats 459 and 462) the dose of atropine administered was 0.15 mgm. per kilogram of body weight. The results obtained in both experiments indicate an apparent increase in the rate of epinephrin output from the adrenals, of approximately 50 per cent.

In the next experiment (cat 458) the dose of atropine was 0.9 mgm. per kilogram of body weight. A few of the tracings used in the assay are given.

Condensed protocol. Cat 458; female (pregnant); weight 2.21 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

10.10 a.m. Cava pocket completed; collected adrenal blood.

10.12½ a.m. First specimen, 0.65 gram in 30 seconds (1.3 grams per minute).

10.13 a.m. Second specimen, 3.7 grams in 2 minutes (1.85 grams per minute); blood pressure 118 mm. of mercury.

10.18½ a.m. End of intravenous injection of 2 mgm. atropine sulphate; blood pressure 110 mm. of mercury.

10.19 a.m. Third specimen, 2.1 grams in 1 minute.

10.20 a.m. Fourth specimen, 3 grams in 2 minutes (1.5 grams per minute); blood pressure 62 mm. of mercury.

10.35 a.m. Stimulation of peripheral end of vagus caused no effect on heart rate or blood pressure.

10.37 a.m. Fifth specimen, 1.5 grams in 1 minute.

10.38 a.m. Sixth specimen, 4.95 grams in 3 minutes (1.65 grams per minute); blood pressure 63 mm. of mercury.

Obtained another specimen of indifferent blood. Combined weight of adrenals 0.395 gram.

The second adrenal specimen, obtained before injection of atropine, was stronger than 1:4,000,000 adrenalin (fig. 4, observations 4 and 8) and weaker than 1:2,700,000 (fig. 4, observation 6). Another observation (not reproduced) showed that the second specimen was somewhat weaker than 1:3,300,000. It was finally assayed at 1:3,500,000 (confirmed by a number of other observations), corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00024 mgm. per kilogram. The third specimen, collected immediately after injection of atropine was much weaker than 1:6,600,000, decidedly stronger than 1:66,000,000 and not unlike 1:20,000,000 (fig. 5, observations 47 and 49), (confirmed by other observations not reproduced). It was taken at 1:20,000,000, corresponding to an output of 0.0001 mgm. per minute for the cat, or 0.00005 mgm. per kilogram. The fourth specimen, obtained 1½ minutes after injection of atropine, was decidedly stronger than 1:13,300,000, stronger than 1:10,000,000, decidedly weaker than 1:4,000,000 and not much

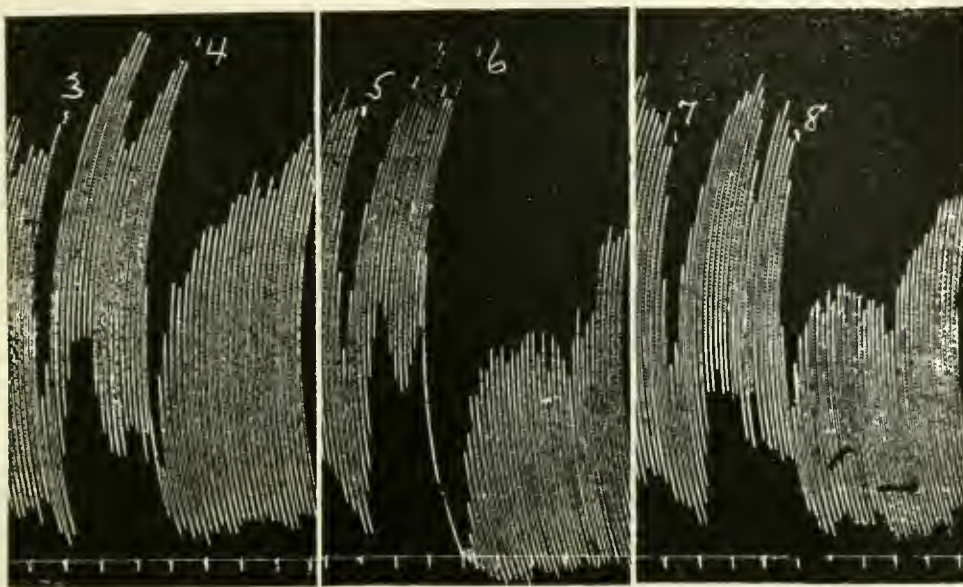


FIG. 4. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 3, 5 and 7, Ringer was replaced by jugular blood and this at 4 by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000; at 6 by jugular blood to which was added adrenalin to make a concentration of 1:2,700,000; at 8 by the second adrenal specimen (collected before intravenous injection of atropine). All the bloods were diluted with 3 volumes of Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

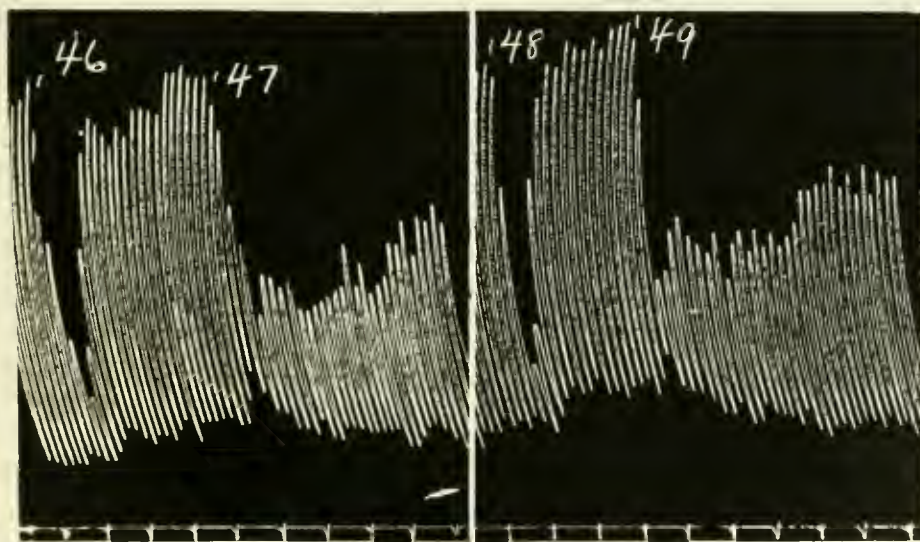


FIG. 5. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 46 and 48, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 47 by the third adrenal specimen (collected immediately after injection of atropine); at 49 by indifferent blood to which was added adrenalin to make a concentration of 1:20,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

different from 1:6,600,000 (fig. 6, observations 37 and 39). The assay at 1:6,600,000 was confirmed by other observations and corresponded to an output of 0.00023 mgm. per minute for the cat, or 0.0001 mgm. per kilogram. The sixth specimen, procured 20 minutes after injection of atropine, was much stronger than 1:4,000,000 adrenalin (fig. 7, observations 23 and 25), stronger than 1:2,700,000 (fig. 7, observation 27) and weaker than 1:1,330,000 (fig. 7, observation 29), (confirmed by three other sets of observations, not reproduced). It was assayed at

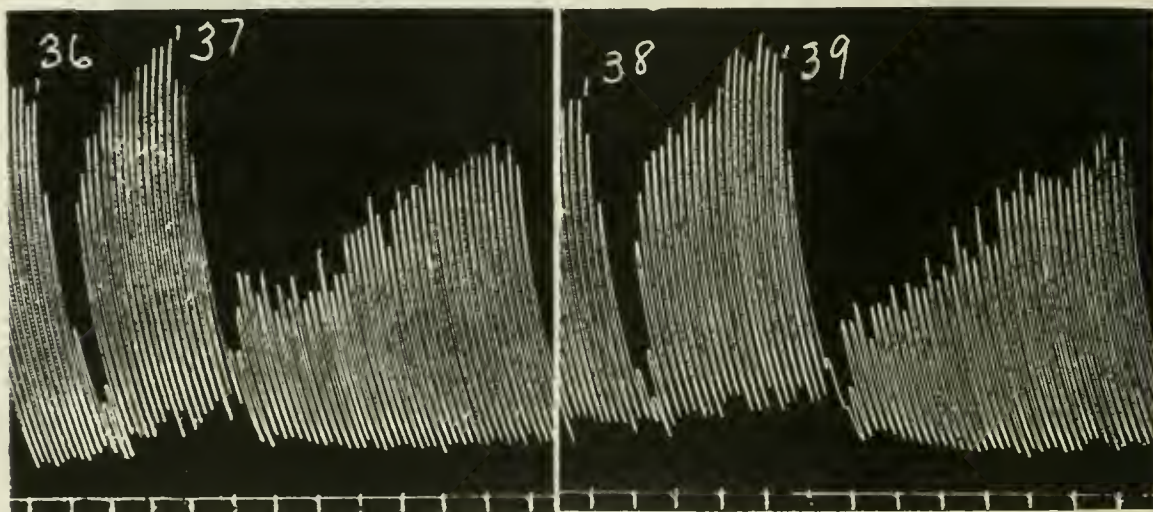


FIG. 6. INTESTINE TRACINGS. BLOODS FROM CAT 45S

At 36 and 38 Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 37 by the fourth adrenal specimen (collected $1\frac{1}{2}$ minutes after intravenous injection of atropine); at 39 by indifferent blood to which was added adrenalin to make a concentration of 1:6,600,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

1:2,500,000, corresponding to an output of 0.0007 mgm. per minute for the cat, or 0.00032 mgm. per kilogram.

The assays of the adrenal blood specimens collected before and after administration of atropine, in this experiment, indicate a transient depression of the epinephrin output during the first few minutes following the injection of the drug. Later, however, while stimulation of the peripheral end of the vagus proved that the drug was still exerting its action on the vagus endings,

the epinephrin output of the adrenals had mounted to somewhat higher than the initial rate.

In the next experiment (cat 461) 10 mgm. of atropine sulphate was administered to a cat weighing 2.43 kgm. (4.1 mgm. per kilogram of body weight). This was the largest dose employed.

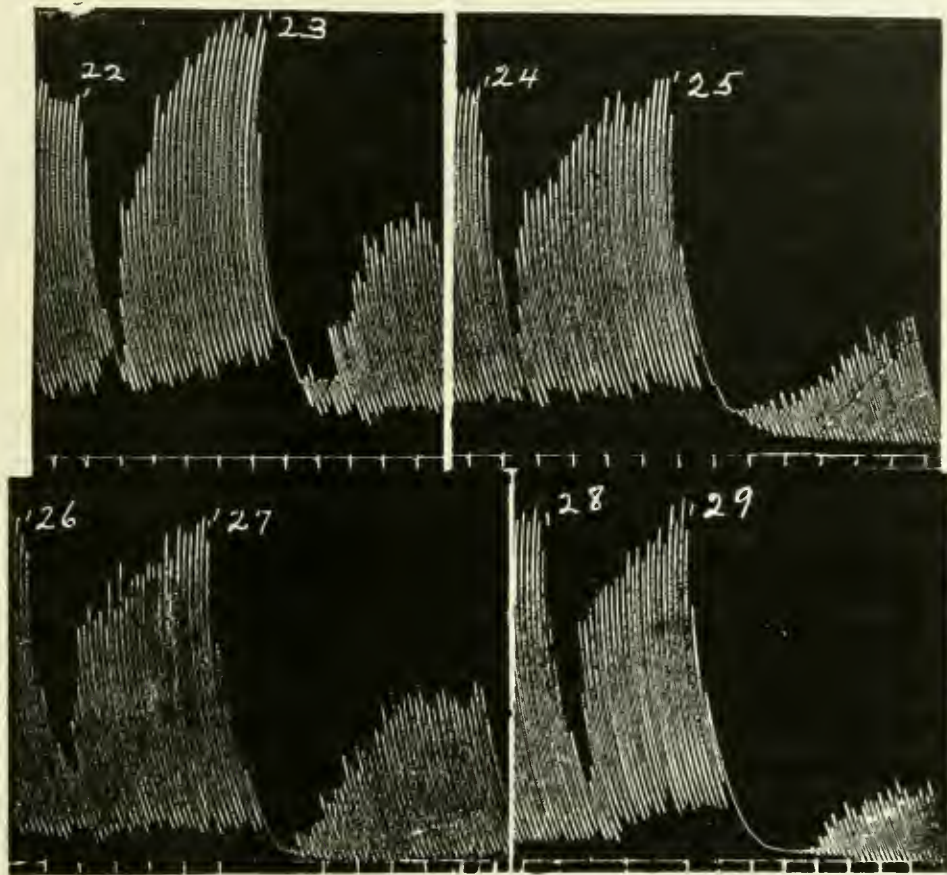


FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 22, 24, 26 and 28, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 23 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000; at 25 by the sixth adrenal specimen (collected 20 minutes after injection of atropine); at 27 by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at 29 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

Condensed protocol. Cat 461; female; weight 2.43 kgm.

Under ether inserted cannulae into the trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

10.10 a.m. Cava pocket completed; collected adrenal blood.

10.12½ a.m. First specimen, 1.45 grams in 30 seconds (2.9 grams per minute).

10.13 a.m. Second specimen, 5.35 grams in 2 minutes (2.7 grams per minute); blood pressure 122 mm. of mercury.

10.25½ a.m. End of intravenous injection of 10 mgm. atropine sulphate; blood pressure 92 mm. of mercury.

10.26 a.m. Third specimen, 1.6 grams in 1 minute.

10.27 a.m. Fourth specimen, 4.5 grams in 3 minutes (1.5 grams per minute); blood pressure 60 mm. of mercury.

10.40½ a.m. Fifth specimen, 0.7 gram in 30 seconds (1.4 grams per minute).

10.41 a.m. Sixth specimen, 4 grams in 3 minutes (1.3 grams per minute); blood pressure 57 mm. of mercury.

Obtained another specimen of indifferent blood. Combined weight of adrenals 0.312 gram.

The assay of the second adrenal specimen, collected before injection of atropine, showed the concentration of epinephrin in the blood to be decidedly less than 1:14,000,000, and somewhat less than 1:28,000,000 (observations not reproduced). It was taken at 1:27,000,000, corresponding to an output of 0.0001 mgm. per minute for the cat, or 0.00004 mgm. per kilogram. This is much below the average output for cats under our experimental conditions.

The fourth specimen, obtained 1½ minutes after injection of atropine, was much weaker than 1:5,700,000 adrenalin, stronger than 1:17,000,000, and not much different from 1:11,400,000, probably slightly weaker (fig. 8, observations 47 and 49). It was assayed at 1:12,000,000 (confirmed by other observations), corresponding to an output of 0.00012 mgm. per minute for the cat, or 0.00005 mgm. per kilogram. The fourth specimen was shown to be stronger than the third in two qualitative observations. Since the blood flow was about the same in both specimens the output during collection of the third specimen must have been somewhat less than during collection of the fourth.

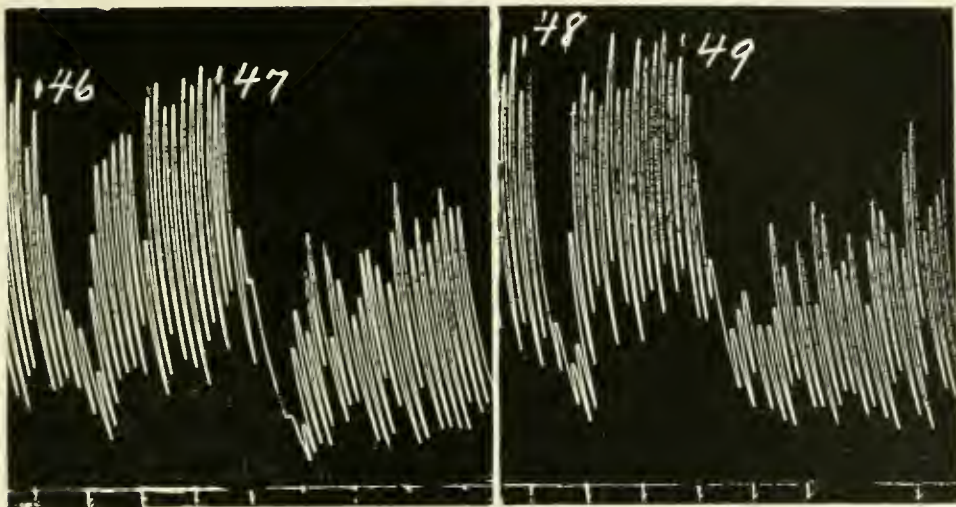


FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT 461

At 46 and 48 Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 47 by indifferent blood to which was added adrenalin to make a concentration of 1:11,400,000; at 49 by the fourth adrenal specimen (collected 1½ minutes after injection of atropine). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fourths.

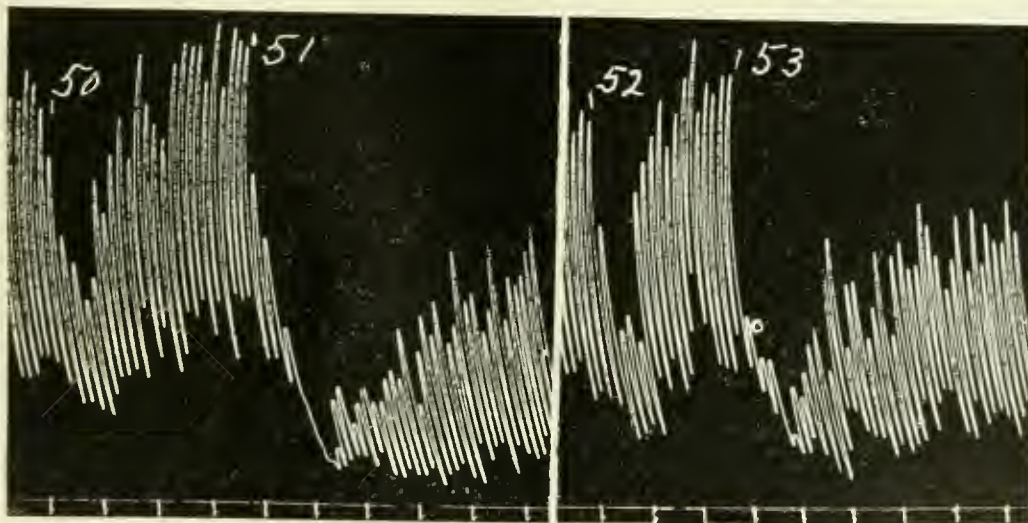


FIG. 9. INTESTINE TRACINGS. BLOODS FROM CAT 461

At 50 and 52, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 51 by indifferent blood to which was added adrenalin to make a concentration of 1:5,700,000; at 53 by the sixth adrenal specimen (collected 15 minutes after injection of atropine). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fourths.

The sixth specimen, procured 15 minutes after injection of atropine, was much stronger than 1:11,400,000 adrenalin, decidedly weaker than 1:4,300,000 and somewhat less than 1:5,700,000 (fig. 9, observations 51 and 53). After confirmation by a number of other observations it was assayed at 1:6,000,000, corresponding to an output of 0.00022 mgm. per minute for the cat, or 0.0001 mgm. per kilogram.

The initial output of adrenal epinephrin, before administration of atropine was unusually low (about one-fourth of the normal average in etherized cats, under our experimental conditions). It is possible that if any depression of the output was caused by the drug it could not manifest itself because of the already very low rate of epinephrin secretion existing. In the sixth specimen, procured 15 minutes after injection of atropine, the epinephrin concentration indicated an output twice as high as the initial rate.

In the next experiment (cat 460) the dose of atropine injected was 1.6 mgm. per kilogram of body weight. The cat had given birth to a litter of kittens six weeks previously. Urethane was used for anesthesia in this experiment, ether in the others.

Condensed protocol. Cat 460; female; weight 3.08 kgm.

Under urethane inserted cannulae into the trachea, external jugular vein, and carotid artery. Obtained indifferent (jugular) blood.

11.00 a.m. Cava pocket completed. Collected adrenal blood.

11.01½ a.m. First specimen, 2.15 grams in 30 seconds (4.3 grams per minute).

11.02 a.m. Second specimen, 5.4 grams in 90 seconds (3.6 grams per minute); blood pressure 116 mm. of mercury.

11.05½ a.m. End of intravenous injection of 5 mgm. atropine sulphate; blood pressure 92 mm. of mercury.

11.06 a.m. Third specimen, 2.65 grams in 1 minute.

11.07 a.m. Fourth specimen, 4.65 grams in 2 minutes (2.3 grams per minute); blood pressure 83 mm. of mercury.

11.20 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (72 mm. of mercury).

11.24 a.m. Fifth specimen, 1.15 grams in 30 seconds (2.3 grams per minute).

11.24½ a.m. Sixth specimen, 4.75 grams in 2 minutes (2.37 grams per minute); blood pressure 72 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.484 gram.

The second adrenal specimen, collected before injection of atropine, was decidedly stronger than 1:4,300,000 adrenalin, stronger than 1:3,550,000, weaker than 1:2,100,000, and not very different from 1:2,850,000. A number of observations confirmed the final assay at 1:2,800,000, corresponding to an output of 0.0013 mgm. per minute for the cat, or 0.0004 mgm. per kilogram. The third specimen, obtained immediately after injection of atropine, was assayed at 1:6,000,000, corresponding to an output of 0.00044 mgm. per minute for the cat, or 0.00014 mgm. per kilogram. The fourth specimen, collected 1½ minutes after injection of atropine, was stronger than 1:5,700,000, and decidedly weaker than 1:2,850,000. The final assay showed it to be equal to 1:4,000,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kilogram. The sixth specimen, obtained 19 minutes after injection of atropine, was decidedly stronger than 1:5,700,000 adrenalin, somewhat stronger than 1:2,850,000, and not quite as strong as 1:2,100,000. It was finally assayed at 1:2,400,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.00032 mgm. per kilogram.

In this animal the initial output of epinephrin from the adrenals, before administration of atropine was higher than the average rate found by us in cats under urethane. There was a moderate transient depression of the epinephrin output, after the injection of atropine, returning to practically the initial rate within 20 minutes. As the rate of output was already high before atropine was injected, it is likely that a moderate increase in the rate that might have been caused by the drug could not be manifested.

Summing up the results of our experiments it will be seen that atropine does not produce a very marked effect on the rate of liberation of epinephrin from the adrenal glands. The drug does not cause complete suppression nor a marked prolonged depres-

sion of the epinephrin output. In fact, the experiments indicate that there may be a moderate increase which, when large doses are employed, may be preceded by a moderate transient depression of the rate of output, under the influence of atropine.

THE INFLUENCE OF PILOCARPINE ON THE EPINEPHRIN OUTPUT

One experiment was made with subcutaneous injection, the others with intravenous administration of pilocarpine. The dose employed was approximately 1 mgm. per kilogram of body weight in all except one cat, which received 0.1 mgm. per kilogram. A number of protocols and some of the tracings used in the assay follow.

Condensed protocol. Cat 287; male; weight 3.715 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein; obtained indifferent (jugular) blood.

12.22 p.m. Cava pocket completed; collected adrenal blood.

12.24 $\frac{1}{4}$ p.m. First specimen, 1.4 grams in 45 seconds (1.9 grams per minute).

12.25 p.m. Second specimen, 4.8 grams in 3 minutes (1.6 grams per minute); blood pressure 92 mm. of mercury.

12.33 p.m. End of intravenous injection of 3 mgm. pilocarpine hydrochloride; blood pressure 72 mm. of mercury. A profuse salivary and lachrymal secretion was evoked, which continued throughout the experiment, and the pupils dilated widely.

12.34 p.m. Third specimen, 2.75 grams in 2 minutes (1.4 grams per minute).

12.36 p.m. Fourth specimen, 3.6 grams in 4 minutes (0.9 gram per minute); blood pressure fell from 76 mm. at beginning of collection to 60 mm. of mercury at end.

Obtained another specimen of jugular blood.

12.50 p.m. Fifth specimen, 0.6 gram in 1 minute.

12.51 p.m. Sixth specimen, 2.6 grams in 5 minutes (0.5 gram per minute); blood pressure 49 mm. of mercury.

Another specimen of indifferent blood was now obtained. Combined weight of adrenals 0.376 gram.

The proportion of serum in the blood was determined by the electrical conductivity method at 44.7 per cent. By the haematocrite, after 5 minutes rotation 30 per cent, after 15 minutes 40 per cent, after 22 minutes 41 per cent and after 30 minutes 42 per cent.

The second adrenal specimen, collected before injection of pilocarpine, was weaker than 1:5,000,000 adrenalin (fig. 10, observations 11 and 13) and somewhat stronger than 1:7,500,000

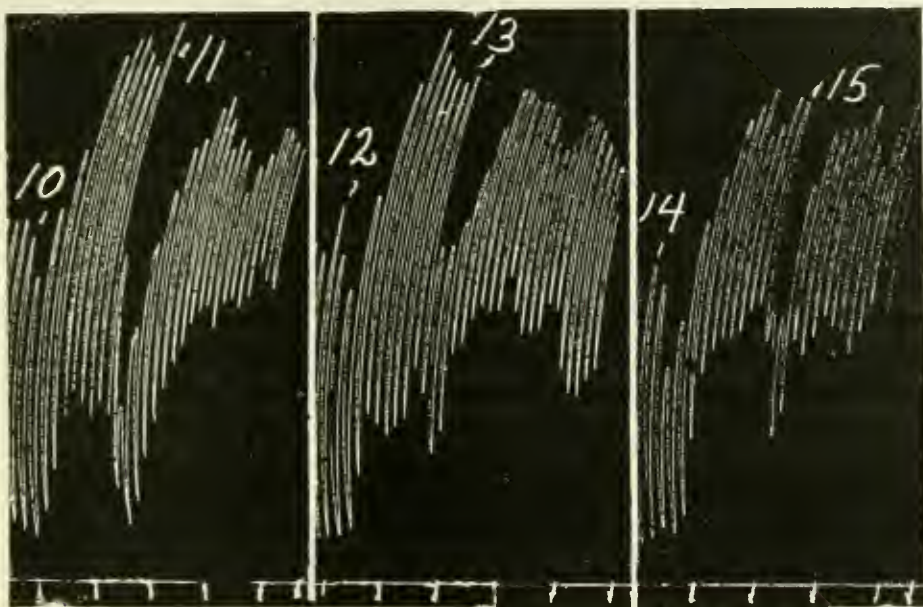


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 287

At 10, 12, and 14, Ringer was replaced by jugular blood and this at 11 by jugular blood to which was added adrenalin to make a concentration of 1:5,000,000; at 13 by the second adrenal specimen (collected before intravenous injection of pilocarpine); at 15 by jugular blood to which was added adrenalin to make a concentration of 1:7,500,000. All the bloods were diluted with 3 volumes of Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fourths.

(fig. 10, observation 15). Another set of observations verified the assay at 1:6,500,000, corresponding to an output of 0.00035 mgm. per minute for the cat, or 0.0001 mgm. per kilogram.

The fourth specimen, obtained 3 minutes after injection of pilocarpine, was decidedly stronger than 1:2,500,000, somewhat stronger than 1:1,300,000 (fig. 11, observations 33, 37 and 39) and decidedly weaker than 1:930,000. It was finally assayed

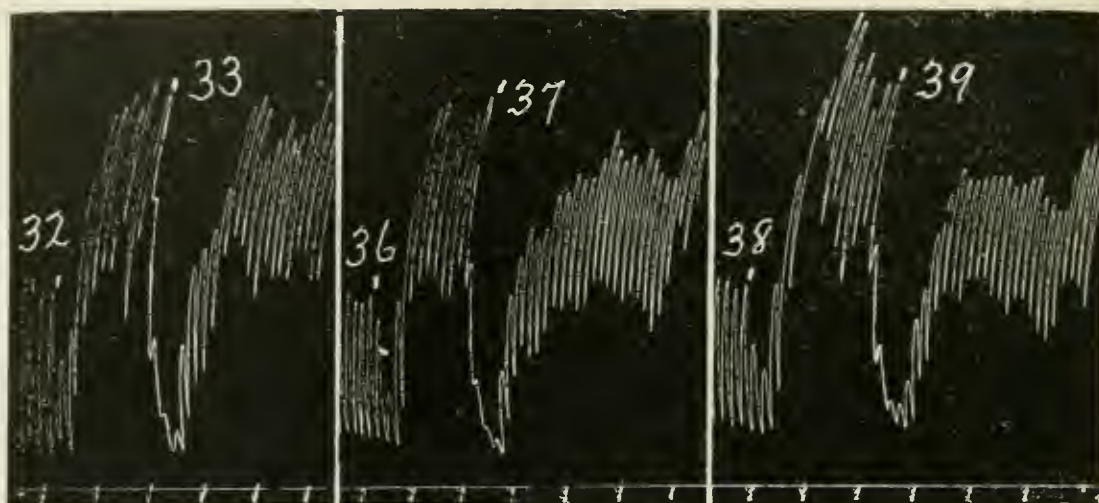


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 287

At 32, 36 and 38, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 33 by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000; at 37 by indifferent blood to which was added adrenalin to make a concentration of 1:1,300,000; at 39 by the fourth adrenal specimen (collected 3 minutes after intravenous injection of pilocarpine). All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

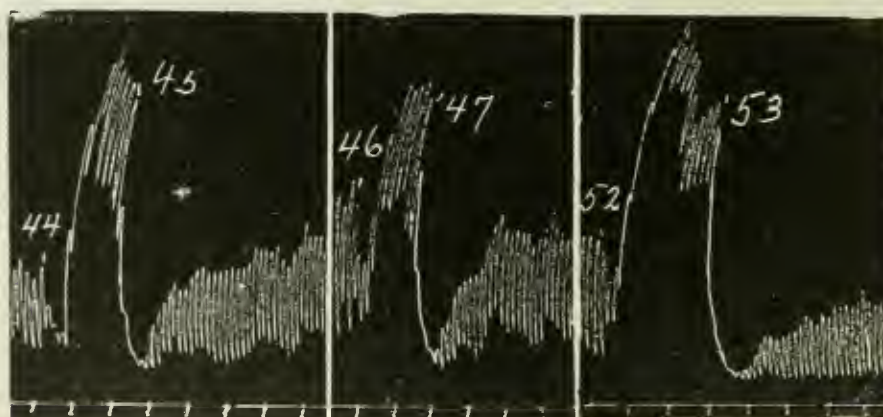


FIG. 12. INTESTINE TRACINGS. BLOODS FROM CAT 287

At 44, 46 and 52, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine); and this at 45 by the sixth adrenal specimen (collected 18 minutes after intravenous injection of pilocarpine); at 47 by indifferent blood to which was added adrenalin to make a concentration of 1:650,000; at 53 by indifferent blood to which was added adrenalin to make a concentration of 1:375,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

at 1:1,200,000 (corroborated by two other sets of observations), corresponding to an output of 0.00075 mgm. per minute for the cat, or 0.0002 mgm. per kilogram.

The sixth specimen, procured 18 minutes after injection of pilocarpine, was shown to be decidedly stronger than 1:875,000 adrenalin, stronger than 1:650,000 (fig. 12, observations 45 and 47), weaker than 1:375,000 (fig. 12, observation 53), and not much different from 1:500,000 (verified by two sets of observations not reproduced). The final assay at 1:500,000 corresponded to an output of 0.001 mgm. per minute for the cat, or 0.00027 mgm. per kilogram. This concentration would correspond to 1:225,000 for the serum, which is somewhat greater than is usually found in the absence of influence of drugs.

The initial rate, before pilocarpine was administered, was somewhat lower than the average output in cats under ether and the drug apparently doubled the output in this animal, although the rate per minute remained within the normal range under our experimental conditions.

Condensed protocol. Cat 289; female, weight 2.83 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein; obtained indifferent (jugular) blood.

11.45 a.m. Cava pocket completed; collected adrenal blood.

11.47 a.m. First specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

11.47½ a.m. Second specimen, 6 grams in 3 minutes (2 gram per minute); blood pressure 97 mm. of mercury.

11.55 a.m. Started artificial respiration.

11.58¼ a.m. End of intravenous injection of 3 mgm. pilocarpine hydrochloride; blood pressure 50 mm. of mercury. Profuse salivary and lachrymal secretion was evoked and pupils dilated widely, which continued throughout the experiment.

11.59 a.m. Third specimen, 2.3 grams in 3 minutes (0.8 gram per minute).

12.02 p.m. Fourth specimen, 3.65 grams in 7 minutes (0.52 gram per minute); blood pressure 44 mm. of mercury.

Another specimen of indifferent blood was now obtained. Combined weight of adrenals 0.342 gram. The proportion of serum in the blood was 72.3 per cent, determined by the electrical conductivity method.

The assay on intestine segments showed that the second adrenal specimen, collected before injection of pilocarpine was much stronger than 1:4,000,000 adrenalin, decidedly stronger than 1:2,700,000, stronger than 1:2,000,000 (fig. 13, observations 21 and 25), and weaker than 1:1,000,000 (fig. 13, observation 23). The final assay (confirmed by a number of other observations) at

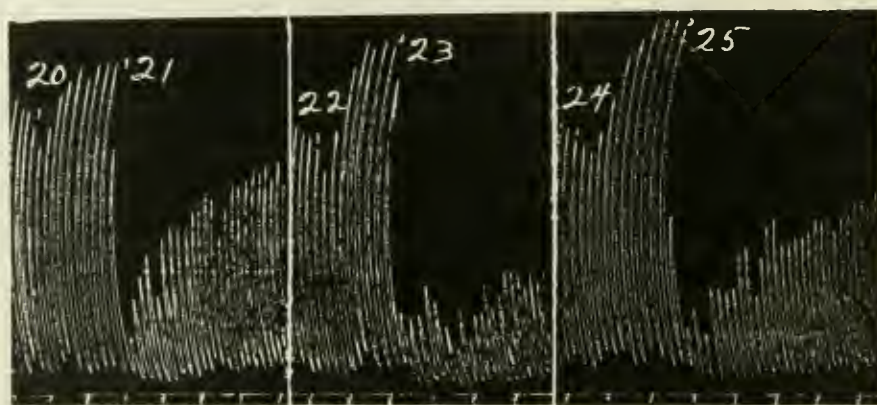


FIG. 13. INTESTINE TRACINGS. BLOODS FROM CAT 289

At 20, 22 and 24, Ringer was replaced by jugular blood and this at 21 by jugular blood to which was added adrenalin to make a concentration of 1:2,000,000 at 23 by jugular blood to which was added adrenalin to make a concentration of 1:1,000,000; at 25 by the second adrenal specimen (collected before intravenous injection of pilocarpine). All the bloods were diluted with 3 volumes of Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

1:1,300,000 corresponded to an output of 0.0015 mgm. per minute for the cat, or 0.0005 mgm. per kilogram. This is greater than the normal average usually found in etherized cats, under our experimental conditions.

The third specimen, obtained immediately after injection of pilocarpine, was decidedly weaker than 1:270,000 (fig. 14, observations 51 and 55), and somewhat stronger than 1:660,000 (fig. 14, observation 53). With the aid of numerous other observations it was assayed at 1:600,000, corresponding to an out-

put of 0.0013 mgm. per minute for the cat, or 0.00046 mgm. per kilogram. The fourth specimen, collected 3 minutes after injection of pilocarpine, was decidedly stronger than 1:660,000 (fig. 14, observations 47 and 53), stronger than 1:400,000 (fig. 14, observation 49) and weaker than 1:270,000 (fig. 14, observation 55). Two other sets of observations (not reproduced) verified

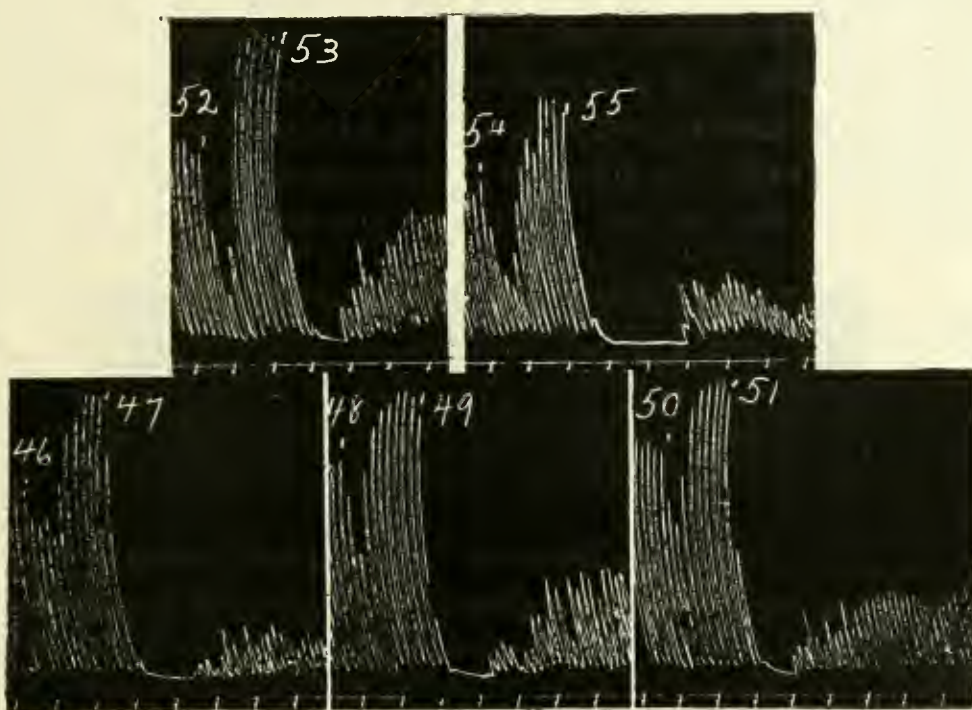


FIG. 14. INTESTINE TRACINGS. BLOODS FROM CAT 289

At 46, 48, 50, 52 and 54, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 47 by the fourth adrenal specimen (collected 3 minutes after injection of pilocarpine); at 49 by indifferent blood to which was added adrenalin to make a concentration of 1:400,000; at 51 by the third adrenal specimen (collected immediately after injection of pilocarpine); at 53 by indifferent blood to which was added adrenalin to make a concentration of 1:660,000; at 55 by indifferent blood to which was added adrenalin to make a concentration of 1:270,000. All the bloods were diluted with 5 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

the assay at 1:350,000, corresponding to an output of 0.0015 mgm. per minute for the cat or 0.0005 mgm. per kilogram. The epinephrin concentration in the fourth specimen was apparently increased beyond the usual maximum under the influence of

pilocarpine and had the blood flows, during collection of the third and fourth specimens, been larger it is possible that a calculated increase in output might have been gotten. It is probable, however, that the drug could not exhibit an increase over the already high initial output existing before injection of pilocarpine.

Qualitative (and frequently quantitative) confirmation of the assays made on segments of intestine are usually obtained with uterus segments. As it is obviously impossible to reproduce all of the tracings used in assays involving a large number of repeated observations, only one figure is given as a sample of the tracings obtained with the uterus. Figure 15 shows that the third adre-

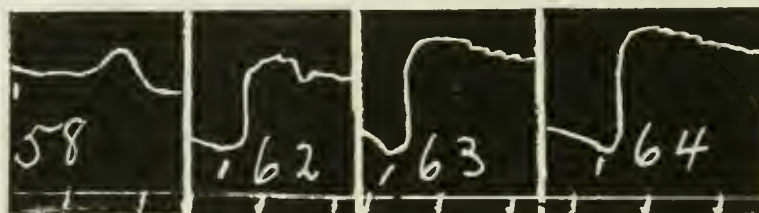


FIG. 15. UTERUS TRACINGS. BLOODS FROM CAT 289

At 58, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine); at 62 by the second adrenal specimen (collected before injection of pilocarpine); at 63 by the third adrenal specimen (collected immediately after injection of pilocarpine); at 64 by the fourth adrenal specimen (collected 3 minutes after injection of pilocarpine). All the bloods were diluted with 6 volumes of Ringer.

nal specimen (observation 63) caused a greater increase in tone than the second (observation 62), and the fourth (observation 64), greater than either of the others. All of the adrenal specimens caused a much larger increase in tone than the corresponding indifferent blood (observation 58). When the epinephrin concentration, in the blood tested, is high, a maximal reaction is ordinarily obtained. For proper qualitative comparisons, therefore, it is usually necessary to dilute the specimen, before applying to the segment, until the reaction obtained is sub-maximal.

Condensed protocol. Cat 463; female; weight 2.87 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.40 a.m. Cava pocket completed; collected adrenal blood.

9.41½ a.m. First specimen, 2.25 grams in 30 seconds (5.5 grams per minute).

9.42 a.m. Second specimen, 5.7 grams in 90 seconds (3.8 grams per minute); blood pressure 84 mm. of mercury.

9.50½ a.m. End of intravenous injection of 3 mgm. pilocarpine hydrochloride; blood pressure 76 mm. of mercury. Profuse salivary and lachrymal secretion, and pupils dilated widely, which continued throughout the experiment.

9.51 a.m. Third specimen, 2.95 grams in 1 minute.

9.52 a.m. Fourth specimen, 7 grams in 2 minutes (3.5 grams per minute); blood pressure 92 mm. of mercury.

10.07½ a.m. Fifth specimen, 1.25 grams in 30 seconds (2.5 grams per minute).

10.08 a.m. Sixth specimen, 6.1 grams in 3 minutes (2 grams per minute); blood pressure 64 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.341 gram.

The second specimen, collected before injection of pilocarpine, was decidedly stronger than 1:13,000,000 adrenalin, much weaker than 1:4,000,000, weaker than 1:5,300,000 and somewhat weaker than 1:6,660,000. Repeated observations confirmed the assay at 1:7,000,000, corresponding to an output of 0.00054 mgm. per minute for the cat, or 0.00019 mgm. per kilogram. The third specimen, collected immediately after injection of pilocarpine, was stronger than 1:6,660,000, and decidedly weaker than 1:2,700,000. It was finally assayed at 1:5,300,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kilogram. The fourth specimen, obtained 1½ minutes after injection of pilocarpine, was stronger than 1:5,300,000, decidedly weaker than 1:2,700,000 and somewhat weaker than 1:4,000,000. The final assay proved it to be equal to 1:4,500,000, corresponding to an output of

0.0008 mgm. per minute for the cat, or 0.00028 mgm. per kilogram.

The sixth specimen, obtained 18 minutes after injection of pilocarpine, was decidedly stronger than 1:5,300,000, stronger than 1:4,000,000, weaker than 1:2,000,000, and not unlike 1:2,700,000. The assay at 1:2,700,000 was verified with a number of observations and corresponded to an output of 0.00075 mgm. per minute for the cat, or 0.00025 mgm. per kilogram.

In the next experiment (cat 464) the animal was anesthetized with urethane.

Condensed protocol. Cat 464: male; weight 2.85 kgm.

Under urethane inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

10.20 a.m. Cava pocket completed; collected adrenal blood.

10.20½ a.m. First specimen, 1.4 grams in 30 seconds (2.8 grams per minute).

10.22 a.m. Second specimen, 4.55 grams in 2 minutes (2.3 grams per minute); blood pressure 97 mm. of mercury.

10.30½ a.m. End of intravenous injection of 4 mgm. pilocarpine hydrochloride; blood pressure 72 mm. of mercury. Profuse salivary and lachrymal secretion evoked and pupils dilated widely, which continued throughout the experiment.

10.31 a.m. Third specimen, 3.5 grams in 1 minute.

10.32 a.m. Fourth specimen, 5.6 grams in 2 minutes (2.8 grams per minute); blood pressure 110 mm. of mercury.

10.45 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (98 mm. of mercury).

10.50 a.m. Fifth specimen, 1.25 grams in 30 seconds (2.5 grams per minute).

10.50½ a.m. Sixth specimen, 4.75 grams in 2½ minutes (1.9 grams per minute); blood pressure 88 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.518 gram.

The second adrenal specimen, obtained before injection of pilocarpine, was decidedly stronger than 1:6,660,000 adrenalin, weaker than 1:4,700,000, decidedly weaker than 1:4,000,000.

Numerous observations showed it to be equal to 1:5,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00015 mgm. per kilogram. The third specimen, collected immediately after injection of pilocarpine, was assayed at 1:20,000,000, corresponding to an output of 0.00017 mgm. per minute for the cat, or 0.00005 mgm. per kilogram. The fourth specimen, obtained $1\frac{1}{2}$ minutes after injection of pilocarpine, was decidedly stronger than 1:6,660,000, weaker than 1:4,000,000, somewhat weaker than 1:5,300,000 and not different from 1:5,000,000. It was finally assayed at 1:5,000,000, corresponding to an output of 0.00056 mgm. per minute for the cat, or 0.0002 mgm. per kilogram. The sixth specimen, obtained 20 minutes after injection of pilocarpine was decidedly weaker than 1:1,300,000, decidedly stronger than 1:5,300,000, much stronger than 1:4,000,000, and not far from 1:2,700,000. It was finally assayed at 1:2,900,000, corresponding to an output of 0.00065 mgm. per minute for the cat, or 0.00022 mgm. per kilogram.

Condensed protocol. Cat 467; female; weight 2.43 kgm.

Under ether inserted cannulae into trachea, external jugular vein, and carotid artery. Obtained indifferent (jugular) blood.

9.55 a.m. Cava pocket completed; collected adrenal blood.

10.00 $\frac{1}{2}$ a.m. First specimen 0.85 grams in 30 seconds (1.7 grams per minute).

10.01 a.m. Second specimen, 3.9 grams in 3 minutes (1.3 grams per minute); blood pressure 100 mm. of mercury.

10.07 $\frac{3}{4}$ a.m. End of intravenous injection of 2.5 mgm. pilocarpine hydrochloride. The pupils dilated widely, and profuse salivary and lachrymal secretion was evoked, lasting throughout the experiment; blood pressure 123 mm. of mercury.

10.08 a.m. Third specimen, 1.95 grams in 1 minute.

10.09 a.m. Fourth specimen, 4.15 grams in $2\frac{1}{2}$ minutes (1.66 grams per minute); blood pressure 118 mm. of mercury.

10.25 a.m. Fifth specimen, 0.45 gram in 30 seconds (0.9 gram per minute).

10.25½ a.m. Sixth specimen, 2.45 grams in 4 minutes (0.6125 gram per minute); blood pressure 56 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.502 gram.

The assay showed that the second adrenal specimen, collected before injection of pilocarpine, was weaker than 1:3,750,000 adrenalin, much stronger than 1:7,500,000, stronger than 1:6,250,000, not far from 1:5,000,000. It was finally assayed at 1:5,500,000, corresponding to an output of 0.00024 mgm. per minute for the cat, or 0.0001 mgm. per kilogram. The third specimen, collected just after injection of pilocarpine, was much stronger than 1:18,750,000, decidedly weaker than 1:10,000,000, and somewhat weaker than 1:12,500,000. It was finally assayed at 1:14,000,000, corresponding to an output of 0.00014 mgm. per minute for the cat, or 0.00006 mgm. per kilogram. The fourth specimen, collected 1½ minutes after injection of pilocarpine, was not quite as strong as 1:7,500,000 and stronger than 1:10,000,000. It was assayed at 1:8,000,000, corresponding to an output of 0.00021 mgm. per minute for the cat, or 0.00009 mgm. per kilogram. The sixth specimen, procured 18 minutes after injection of pilocarpine, was much stronger than 1:3,750,000 adrenalin, stronger than 1:2,500,000, and not very different from 1:2,000,000. It was assayed at 1:2,000,000, corresponding to an output of 0.0003 mgm. per minute for the cat, or 0.00014 mgm. per kilogram.

In the next experiment (cat 465), a much smaller dose of pilocarpine was administered (0.1 mgm. per kilogram of body weight).

Condensed protocol. Cat 465; female; weight 2.86 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.45 a.m. Cava pocket completed; collected adrenal blood.

9.46½ a.m. First specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

9.47 a.m. Second specimen, 4.25 grams in 2 minutes (2.1 grams per minute); blood pressure 124 mm. of mercury.

- 10.10 $\frac{1}{2}$ a.m. End of intravenous injection of 0.3 mgm. pilocarpine hydrochloride; blood pressure 94 mm. of mercury. The pupils dilated widely and profuse salivary and lachrymal secretion was evoked, lasting throughout the experiment.
- 10.11 a.m. Third specimen, 3.8 grams in 1 minute.
- 10.12 a.m. Fourth specimen, 6.85 grams in 2 minutes (3.4 grams per minute); blood pressure 104 mm. of mercury.
- 10.20 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (94 mm. of mercury).
- 10.25 a.m. Fifth specimen, 1.3 grams in 30 seconds (2.6 grams per minute).
- 10.25 $\frac{1}{2}$ a.m. Sixth specimen, 6.2 grams in 3 minutes (2.1 grams per minute); blood pressure 78 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.405 gram.

The epinephrin concentration in the second specimen, obtained before injection of pilocarpine, was decidedly greater than 1:6,250,000, greater than 1:5,000,000 (fig. 16, observations 14 and 16), much less than 1:3,125,000, and not as great as 1:3,750,000 (fig. 16, observation 12). It was finally assayed at 1:4,300,000 (corroborated by four sets of observations), corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00018 mgm. per kilogram. The concentration of epinephrin in the third specimen, collected immediately after injection of pilocarpine, was approximately the same as that in the fourth specimen, collected 1 $\frac{1}{2}$ minutes after injection of pilocarpine (fig. 17, observations 26 and 28). Both specimens were somewhat stronger than 1:7,500,000 (fig. 17, observation 32), and weaker than 1:6,250,000 (fig. 17, observation 34). A number of other observations verified the assay at 1:7,000,000, corresponding to an output of 0.00054 mgm. per minute for the cat, or 0.00019 mgm. per kilogram for the third specimen and 0.00048 mgm. per minute for the cat, or 0.00017 mgm. per kilogram for the fourth specimen. The sixth specimen, collected 15 minutes after injection of pilocarpine, was decidedly stronger than 1:9,375,000, stronger than 1:7,500,000, weaker than 1:5,000,000, and somewhat weaker than

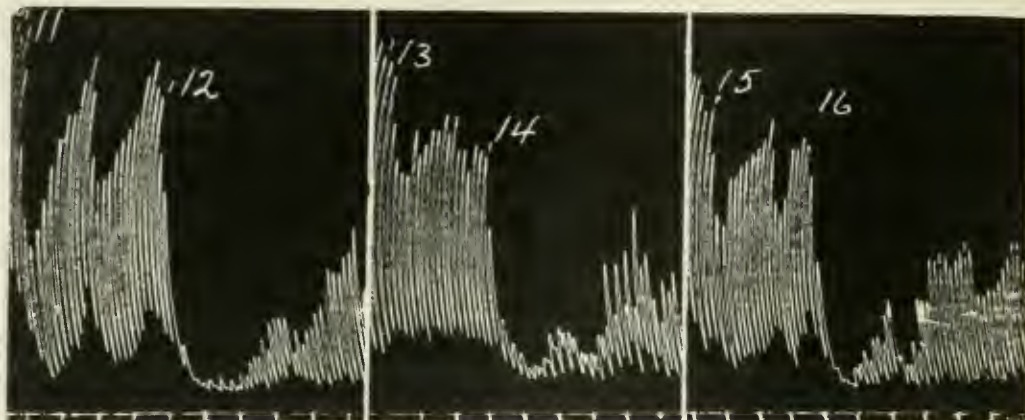


FIG. 16. INTESTINE TRACINGS. BLOODS FROM CAT 465

At 11, 13 and 15, Ringer was replaced by jugular blood and this at 12 by jugular blood to which was added adrenalin to make a concentration of 1:3,750,000; at 14 by the second adrenal specimen (collected before intravenous injection of pilocarpine); at 16 by jugular blood to which was added adrenalin to make a concentration of 1:5,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

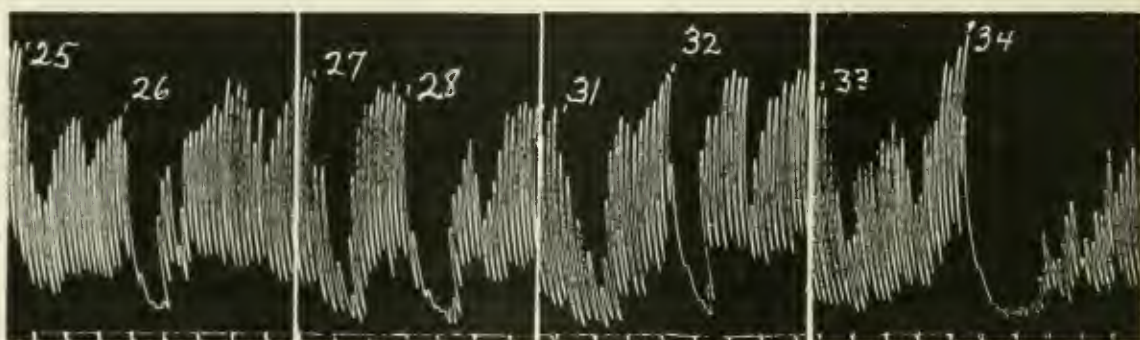


FIG. 17. INTESTINE TRACINGS. BLOODS FROM CAT 465

At 25, 27, 31 and 33, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 26 by the fourth adrenal specimen (collected 1½ minutes after injection of pilocarpine); at 28 by the third adrenal specimen (collected immediately after injection of pilocarpine); at 32 by indifferent blood to which was added adrenalin to make a concentration of 1:7,500,000; at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:6,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

1:6,250,000 (fig. 18 observations 36 and 38). It was finally assayed at 1:6,500,000 (corroborated by three other sets of observations), corresponding to an output of 0.00032 mgm. per minute for the cat, or 0.00011 mgm. per kilogram.

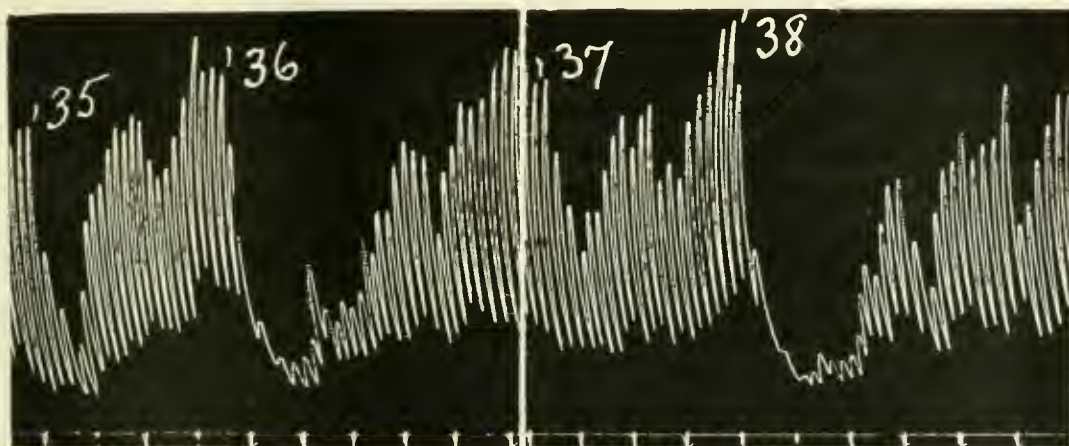


FIG. 18. INTESTINE TRACINGS. BLOOD FROM CAT 465

At 35 and 37, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 36 by the sixth adrenal specimen (collected 15 minutes after injection of pilocarpine); at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:6,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fourths.

In the following experiment, pilocarpine was administered subcutaneously.

Condensed protocol. Cat 466; female; weight 2.77 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.40 a.m. Cava pocket completed; collected adrenal blood.

9.45½ a.m. First specimen, 2.2 grams in 30 seconds (4.4 grams per minute).

9.46 a.m. Second specimen, 5.65 grams in 90 seconds (3.8 grams per minute); blood pressure 114 mm. of mercury.

9.53 to 9.54 a.m. Subcutaneous injection of 3 mgm. pilocarpine hydrochloride. The pupils dilated widely and profuse salivary and lachrymal secretion was evoked lasting throughout the experiment; blood pressure at end of injection 90 mm. of mercury.

- 9.54 a.m. Third specimen, 3.5 grams in 1 minute.
9.55 a.m. Fourth specimen, 5.2 grams in 2 minutes (2.6 grams per minute); blood pressure 75 mm. of mercury.
10.02 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (67 mm. of mercury).
10.10 a.m. Fifth specimen, 0.7 gram in 30 seconds (1.4 grams per minute).
10.10½ a.m. Sixth specimen, 3.1 grams in 3 minutes (1 gram per minute); blood pressure 47 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.277 gram.

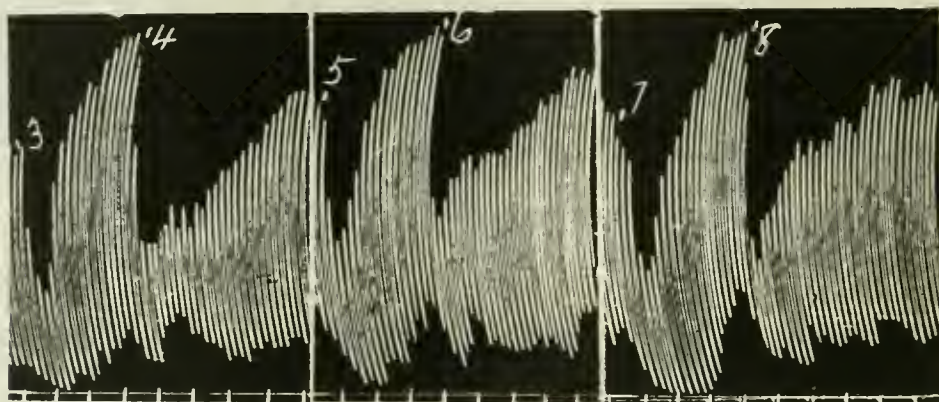


FIG. 19. INTESTINE TRACINGS. BLOODS FROM CAT 466

At 3, 5 and 7, Ringer was replaced by jugular blood and this at 4 by jugular blood to which was added adrenalin to make a concentration of 1:6,660,000; at 6 by jugular blood to which was added adrenalin to make a concentration of 1:13,300,000; at 8 by the second adrenal specimen (collected before subcutaneous injection of pilocarpine). All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

The second adrenal specimen, obtained before injection of pilocarpine, was decidedly less than 1:5,300,000, less than 1:6,660,000 (fig. 19, observations 4 and 8), and greater than 1:13,300,000 (fig. 19, observation 6). The final assay was confirmed by a number of other observations at 1:9,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00015 mgm. per kilogram.

The fourth specimen, collected 1 minute after injection of pilocarpine, was stronger than 1:5,300,000 adrenalin (fig. 20,

observations 52 and 54), and not quite as strong as 1:4,000,000. It was assayed at 1:4,600,000 (corroborated by two sets of observations), corresponding to an output of 0.00055 mgm. per minute for the cat, or 0.0002 mgm. per kilogram.

The sixth specimen, procured 16 minutes after injection of pilocarpine, was decidedly stronger than 1:2,700,000, somewhat stronger than 1:2,250,000 adrenalin (fig. 21, observations 46 and 48) and weaker than 1:1,330,000 (fig. 21, observation 50).

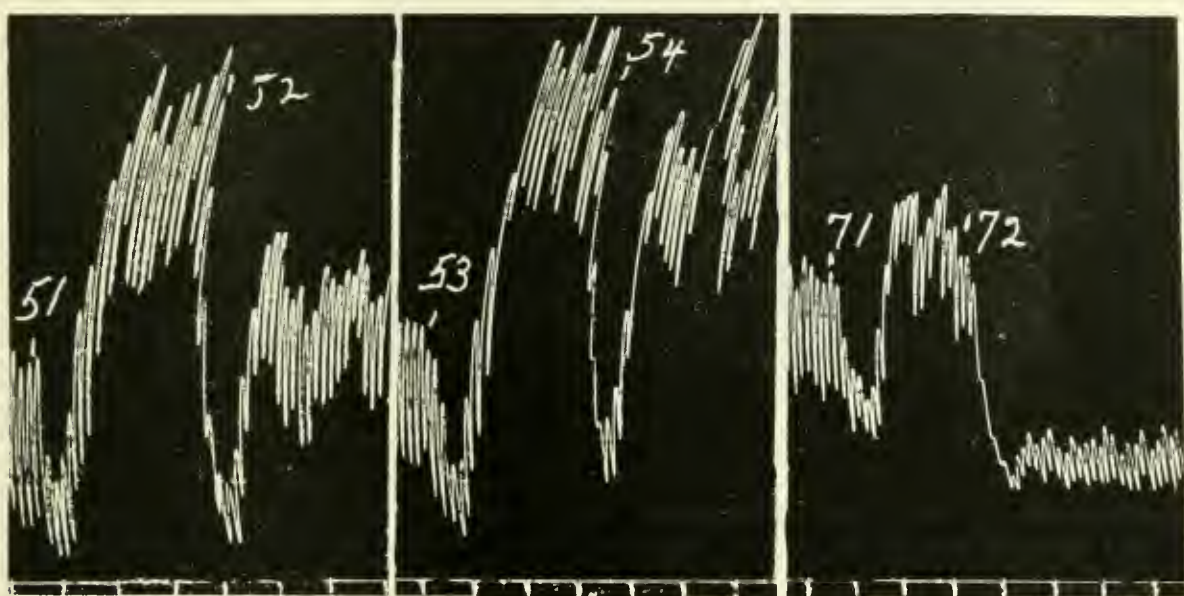


FIG. 20. INTESTINE TRACINGS. BLOODS FROM CAT 466

At 51, 53 and 71, Ringer was replaced by indifferent blood (collected after subcutaneous injection of pilocarpine) and this at 52 and at 72 by the fourth adrenal specimen (collected 1 minute after injection of pilocarpine); at 54 by indifferent blood to which was added adrenalin to make a concentration of 1:5,300,000. Before observation 71-72 the segment had been subjected to the action of atropine. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fourths.

Three other sets of observations corroborated the assay at 1:2,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat or 0.00018 mgm. per kilogram.

A very interesting point in technique is illustrated in figure 20, observations 52 and 72. Both tracings were obtained with the same segment of intestine, subjected to the action of the same adrenal blood (fourth specimen), at 52 before atropinizing and

at 72 after atropine had been applied to the segment. We have repeatedly observed that subjecting the segment of intestine to the action of atropine renders it much more sensitive to the action of epinephrin and that specimens which have an epinephrin concentration too low to cause a definite inhibition of tone (or suppression of beats) before atropine is applied, cause a good inhibition after atropinizing the segment, or where a good reaction was obtained before atropine was used a much greater inhi-

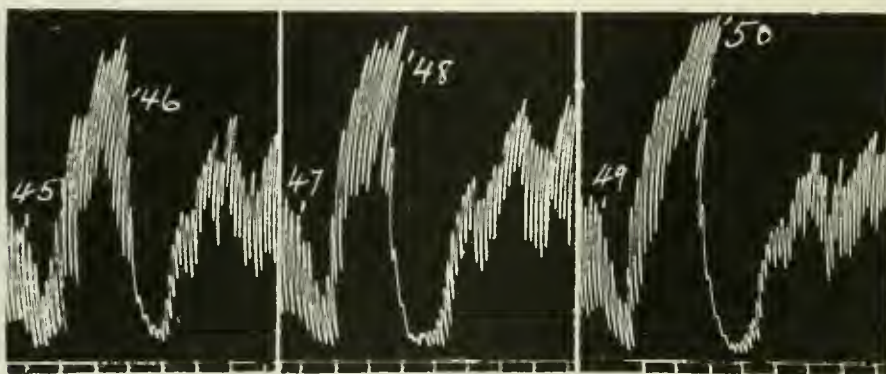


FIG. 21. INTESTINE TRACINGS. BLOODS FROM CAT 466

At 45, 47, and 49, Ringer was replaced by indifferent blood (collected after subcutaneous injection of pilocarpine); and this at 46 by indifferent blood to which was added adrenalin to make a concentration of 1:2,250,000; at 48 by the sixth adrenal specimen (collected 16 minutes after subcutaneous injection of pilocarpine); at 50 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

bition (and suppression of beats) is caused by the same specimen after the application of atropine as illustrated in figure 20. Generally, although not always, the first application of atropine causes a temporary inhibition of the contractions. The beats usually become smaller, but frequently increase after a few applications of blood to the same magnitude as before the atropine.

In nearly all of the experiments with pilocarpine, the adrenal blood specimens were first assayed on the non-atropinized segment, then again on the same segment after subjecting it to the influence of atropine.

THE INFLUENCE OF PILOCARPINE ON THE EPINEPHRINE STORE OF THE ADRENALS

One experiment was made (cat 302) on the influence of pilocarpine on the store of epinephrin in the adrenals. The left adrenal had been denervated and the left superior cervical ganglion excised two weeks previous to the experiment. Thirty-one milligrams of pilocarpine hydrochloride was injected (in doses of 5 to 10 mgm.) within a period of 5 hours. The glands were assayed colorimetrically by the method of Folin, Cannon and Denis (12). The condensed protocol of the experiment follows.

Condensed protocol. Cat 302; female; weight 1.65 kgm.

Left pupil contracted and nictitating membrane forward. Left adrenal denervated and left superior cervical ganglion excised two weeks previously.

11.15 a.m. Injected 5 mgm. pilocarpine hydrochloride subcutaneously.

11.20 a.m. Salivation; no change in pupils.

11.25 a.m. Injected 5 mgm. pilocarpine hydrochloride.

11.30 a.m. Vomited; both pupils dilated widely, the left more than the right; both nictitating membranes retracted (remained retracted throughout the rest of the experiment). Considerable salivation.

11.40 a.m. Right pupil $\frac{2}{3}$ to $\frac{3}{4}$ dilated, left pupil dilated to maximal.

12.50 p.m. Pupils equal (about $\frac{3}{4}$ dilated).

1.19 p.m. Injected 5 mgm. pilocarpine hydrochloride.

1.23 p.m. Vomited; same effect on pupils as noted in observation of 11.30.

2.40 p.m. Pupils equal (about $\frac{2}{3}$ dilated). Injected 6 mgm. pilocarpine hydrochloride.

3.05 p.m. Vomited; transitory dilatation of both pupils, the left becoming wider than right, returning to equality in a few minutes; salivation and lachrymation pronounced.

4.00 p.m. Injected 10 mgm. pilocarpine hydrochloride. The injection caused the cat to become violently angry, and bit its attendant; during this rage both pupils dilated widely the left becoming maximal and the right nearly so. As soon as the cat became pacified the pupils returned to equality.

4.50 p.m. Pupils equal (about $\frac{3}{4}$ dilated) and nictitating membranes still retracted. The cat was now killed by a sudden blow on the head.

The left adrenal weighed 0.25 gram and contained 0.31 mgm. epinephrin. The right adrenal weighed 0.25 gram and contained 0.24 mgm. epinephrin.

There was no marked depletion of the epinephrin store in the unprotected (right) gland as compared with the denervated (left) gland. There was, however, a small but definite difference between the two glands (0.31 mgm. in the left, and 0.24 mgm. in the right). Elliott (13) reported that he failed to find proof that pilocarpine influences the adrenalin load in any way. He quotes two experiments in which the pilocarpine was administered to cats under ether. In the first experiment the protected gland contained 0.26 mgm. epinephrin and the other 0.17 mgm. In the second experiment the protected gland contained 0.30 mgm. epinephrin and the other 0.24 mgm. (assayed by the blood pressure method). In Elliott's experiments it would be impossible to separate the effect of ether on the epinephrin store from that of the pilocarpine. For he has shown, and we have confirmed his observation (14) that ether causes a diminution in the store. In our experiment the cat was not anesthetized, the drug being administered subcutaneously. The effect produced by pilocarpine on the store is apparently much less than that caused by some other drugs (morphine, β -tetra-hydronaphthylamine).

Our experiments with pilocarpine indicate that the rate of output of epinephrin from the adrenals is not materially altered under the influence of this drug. In only one experiment (cat 287) was the output apparently increased to about double the initial rate before pilocarpine was injected, and this increase only brought the output up to the ordinary average rate found by us in the absence of influence of drugs, the initial rate being somewhat lower than the average. The only other evidence of a possible action exerted by this drug was obtained in cat 289. The high epinephrin concentration found in the fourth specimen (blood flow 0.52 gram per minute) may indicate that, under certain

conditions, pilocarpine is probably capable of increasing the concentration of epinephrin in adrenal vein blood beyond the normal possible maximum usually found in specimens collected with very slow blood flows. Whatever action, if there is any genuine action on the epinephrin secretion from the adrenals, that is caused by pilocarpine, it is certainly not comparable to the pronounced effect caused by this drug on the secretion of saliva, sweat, etc.

SUMMARY

1. The rate of output of epinephrin from the adrenals (in cats) is not materially influenced by the action of atropine or pilocarpine. A moderate increase in the rate of liberation may be produced by atropine, which in large doses may be preceded by a moderate transient depression of the rate of output. The augmentation of the output, if any is caused, by pilocarpine is small and is not comparable with the large increase in the rate of epinephrin liberation caused by strychnine or the immediate effect of nicotine.

2. One experiment was performed which indicates that pilocarpine is capable of producing a moderate depletion of the epinephrin store, in adrenal glands with intact nerve supply.

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A STUDY OF THE ACTION OF COCAINE ON THE SPLANCHNIC AND CERVICAL SYMPATHETIC NEUROMUSCULAR MECHANISMS

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A synergistic action between cocaine and epinephrine has been recognized since the work by Loewi and Froelich (1). This synergism is readily demonstrated by observing the more prolonged and more powerful vasoconstriction produced by epinephrine on intravenous injection at the same time as or following shortly after an injection of a very small amount of cocaine. The same synergistic action apparently occurs in pupillary reactions to epinephrine following cocaine administration. The principle is so widely accepted in clinical practice of medicine that reiteration of these facts might appear superfluous. Yet it is an uncertain step to assume that because such a synergism exists it must of necessity follow that cocaine sensitizes the peripheral sympathetic neuromuscular mechanism to any form of stimulus, for it is conceivable that the two drugs so influence one another as to be more effective when given together without the existence of any fundamental changes in the mechanism itself towards normal or other types of nerve stimulation. This question appears more serious when one considers the trend of modern thought towards the recognition of a rather wide disparity between the pharmacodynamic action of epinephrine as a drug and the demonstrated physiology of the adrenal glands and their secretion.

Kuroda (2), in making a study of cocaine on various tissues innervated by the sympathetic system, blood vessels, uterus, intestine and urinary bladder, came to the conclusion that co-

caine had no action on such structures comparable to those provoked by epinephrine, but whatever action did occur was the result of direct action of the drug upon smooth muscle fibers, first weakly stimulating and later or if in greater concentration paralyzing them. He failed to find any other feasible explanation of the effects of cocaine on the iris than direct muscle action. It is evident thus that Kuroda's experimental results which of themselves need not be disputed, are not so designed as to reveal any other point or mode of action of small amounts of cocaine. The fact that local installation of epinephrine (1:1000) onto the eye-ball of a normal rabbit produces no mydriasis while after cocaine marked mydriasis occurs indicates a striking instance of synergism of these two drugs, should have suggested that cocaine so sensitizes the mechanism that epinephrine becomes effective. This is scarcely consistent with the view Kuroda holds that cocaine produces mydriasis solely by circular muscle depression. Furthermore the synergism of cocaine and epinephrine in blood pressure and perfusion experiments (Froelich and Loewi (1), Fischel (3)) are not consistent with the idea of muscle depression unless in such a state of depression the muscle thereby becomes more susceptible to at least one adequate stimulus, namely, epinephrine. In these conditions the muscular mechanism actually becomes more powerful than before being affected by cocaine.

Pilocarpine, muscarine and physostigmine contract the cocaineized pupil consequently the paralysis is not seriously in evidence. Cushny (4) states that aside from the iris "cocaine differs from adrenalin in not affecting the sympathetic fibers in any other organs."

v. Anrep (5) states that division of the splanchnic nerve during the short rise of pressure caused by cocaine injection causes a fall while stimulation of the splanchnic nerve causes a rise. With greater doses no rise was obtained neither did splanchnic stimulation evoke any response. We believe such experiments were inadequate to demonstrate the early action of cocaine for he appeared to have made no comparative observations on irritability of the nerve before and after sufficiently small doses.

doses which in themselves would not be sufficient to paralyze the smooth neuromuscular mechanism.

Sollmann (6) states that "cocaine increases the peripheral excitability of the sympathetic nervous system without being directly stimulant." For this statement he gives no reference nor do I find any definite proof in the literature directly supporting this idea aside from indirect evidences from simultaneous use of cocaine and epinephrine as an exciting agent. While this may be logical reasoning in this particular instance it is by no means proof that cocaine exerts an action independent of the simultaneous activity of epinephrine.

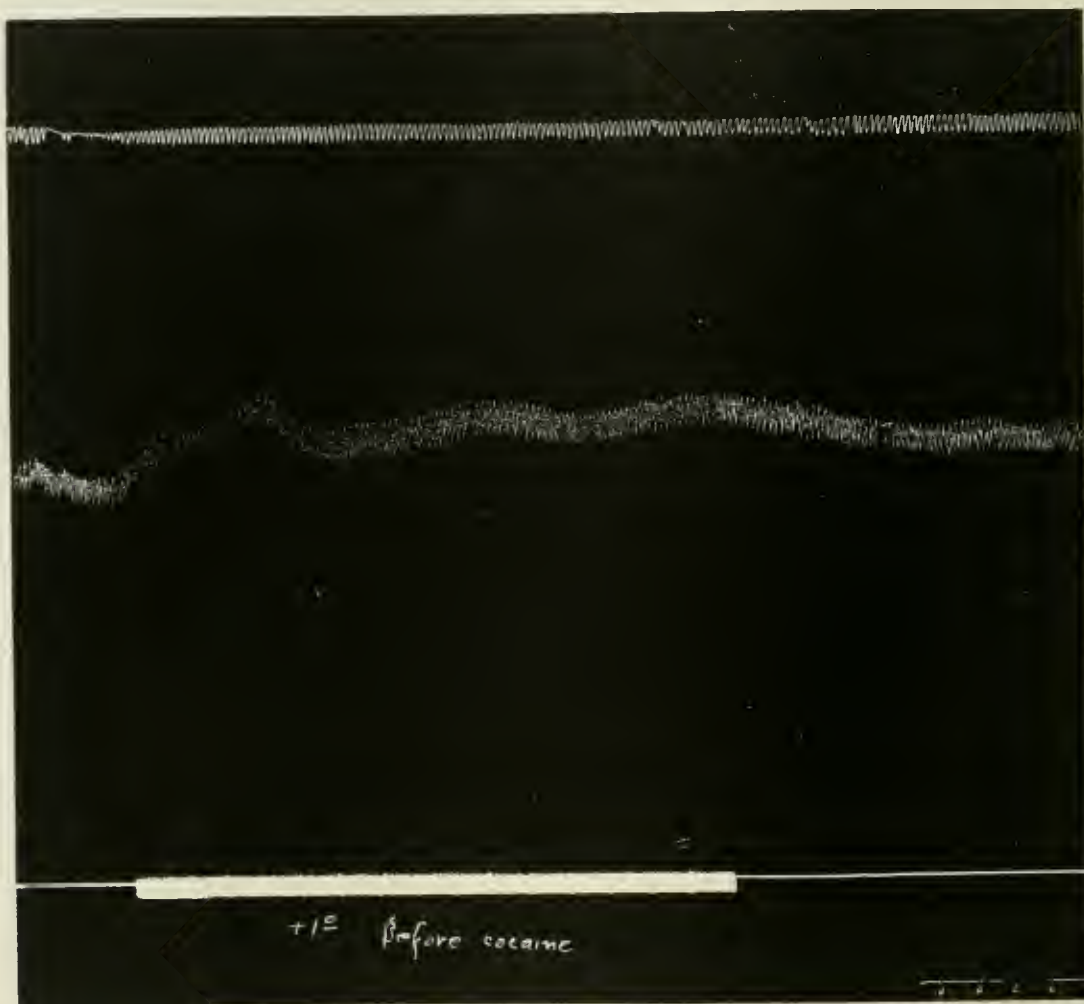
In this connection it may be stated that Niwa (7) using the Tashiro biometer found cocaine in preanesthetic concentrations to cause an increase in carbon dioxide output from isolated nerve preparations. We do not necessarily infer that the nerve fiber alone should have taken part in any change in excitability but rather we consider such results to indicate the existence of an increased irritability probably involving more extensively the whole or greater part of the neuromuscular mechanism in question. Pointing in this direction also are the observations of Carlson (8) on the action of cocaine on the ganglia of the limulus heart.

The primary object of this paper is, then, to ascertain if possible whether the augmentation of sympathetic activity is a condition of increased neuromuscular irritability responding more powerfully to other types of stimuli or whether it is limited to epinephrine as an exciting agent. For purposes of constancy of stimulation energy the induction current was used, though theoretically any other form might equally well have been chosen.

EFFECT OF COCAINE ON THE SPLANCHNIC SYSTEM

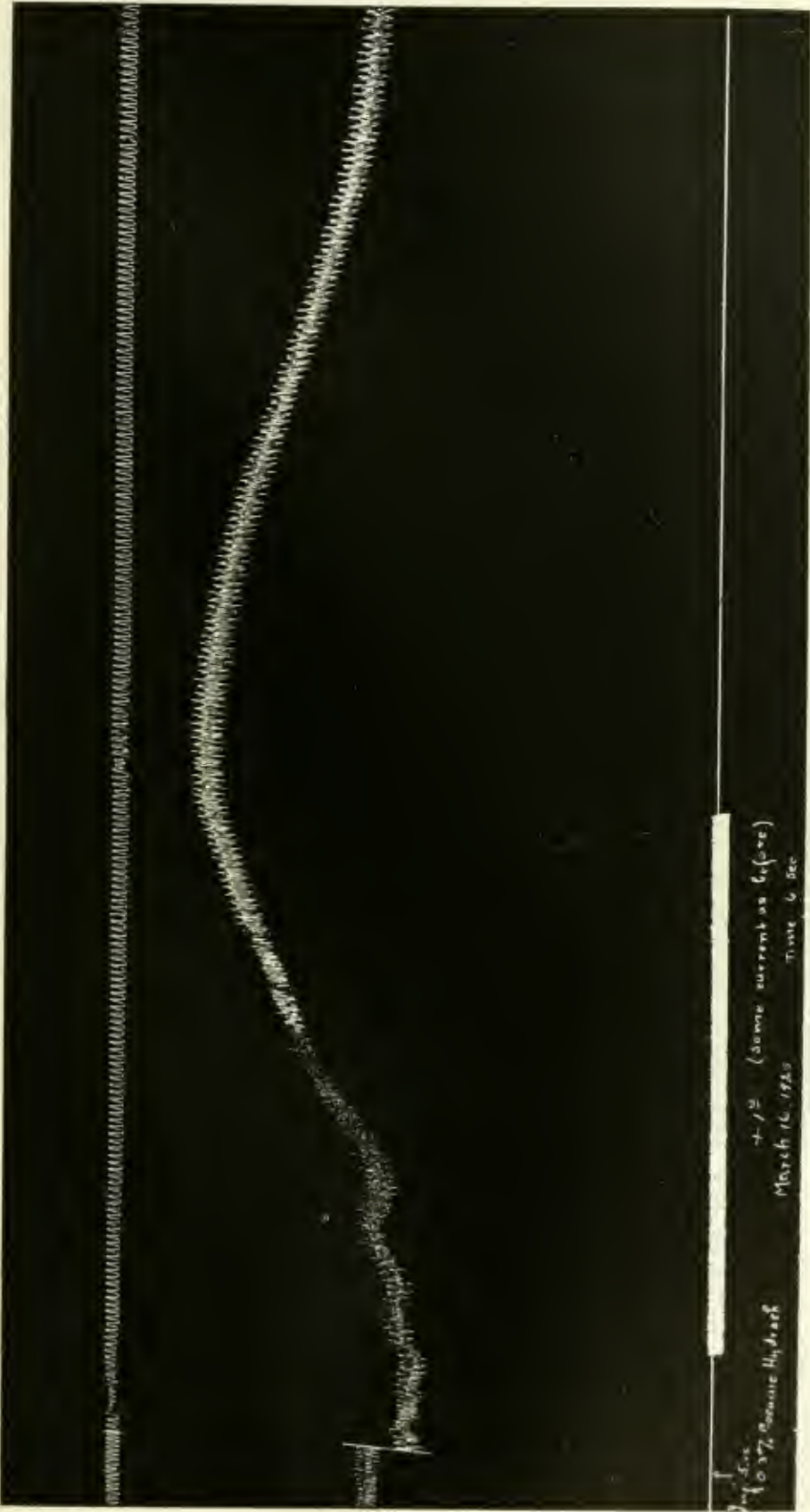
Since the splanchnic area constitutes the great reservoir of blood and its vasomotor control the chief point of changes in blood pressure as a whole, this system was first studied. After isolation of one splanchnic nerve in the dog under ether anesthesia blood pressure tracings were taken with near minimal strengths

of current applied to electrodes attached to the splanchnic nerve. This minimal effective current being established for one particular preparation was maintained throughout, or in certain instances weakened, depending on magnitude of pressure changes rather than changes in threshold of irritability.



TRACING I. BLOOD PRESSURE TRACING. DOG. ETHER ANESTHESIA
Effect of splanchnic nerve stimulation by weak faradic current. Control.

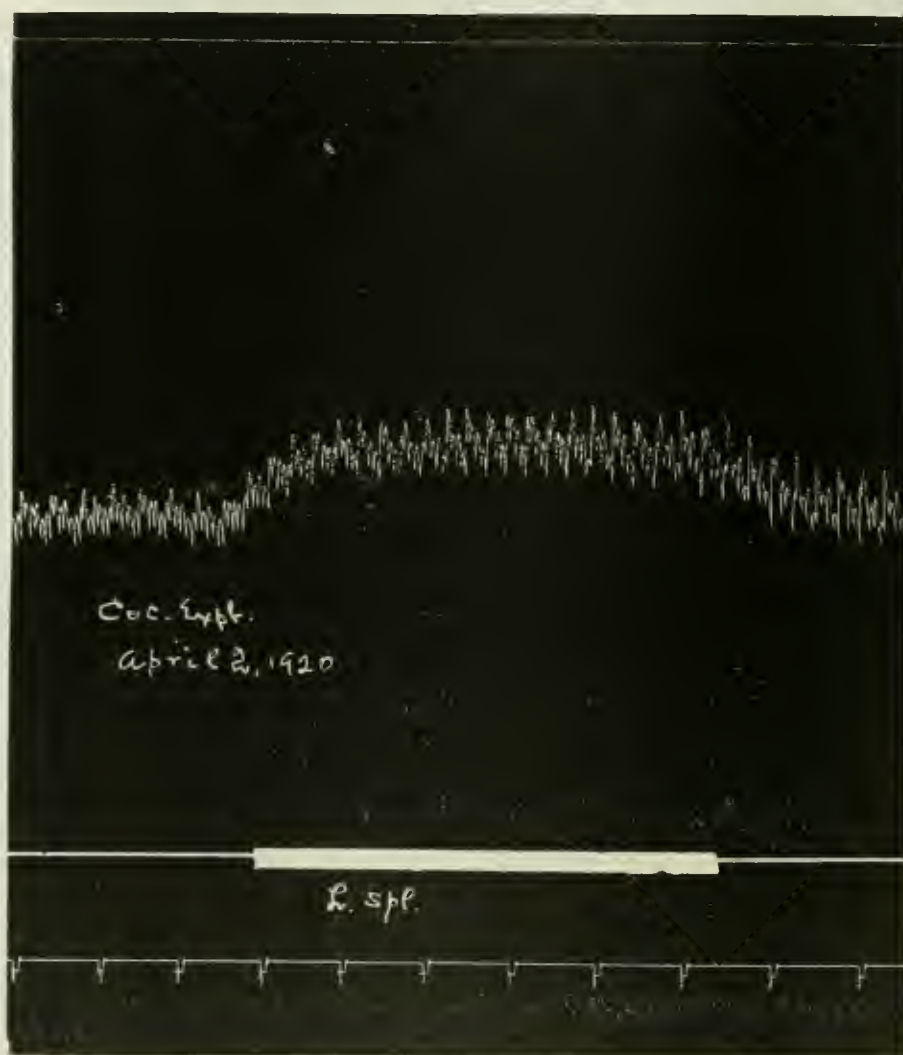
After cocaine injections into the femoral vein, the same stimulus produced a remarkably augmented blood pressure response. Both height of pressure and duration of response were greatly increased. This augmentation however is obviously not necessarily purely one of vasomotor nerve changes in irritability, for the reason that the nerves were stimulated at a point proximal



TRACING II. BLOOD PRESSURE TRACING. DOG. ETHER ANESTHESIA

Effect of stimulation of the same nerve with the same strength of current after a femoral vein injection of 5 cc. of 0.3 per cent solution of cocaine hydrochloride.

to the adrenal glands; consequently the augmentation of response to electrical stimulation might after all be chemical in origin, owing to outpouring of epinephrine which in turn may synergize with cocaine. This in fact appears to be true since the curve



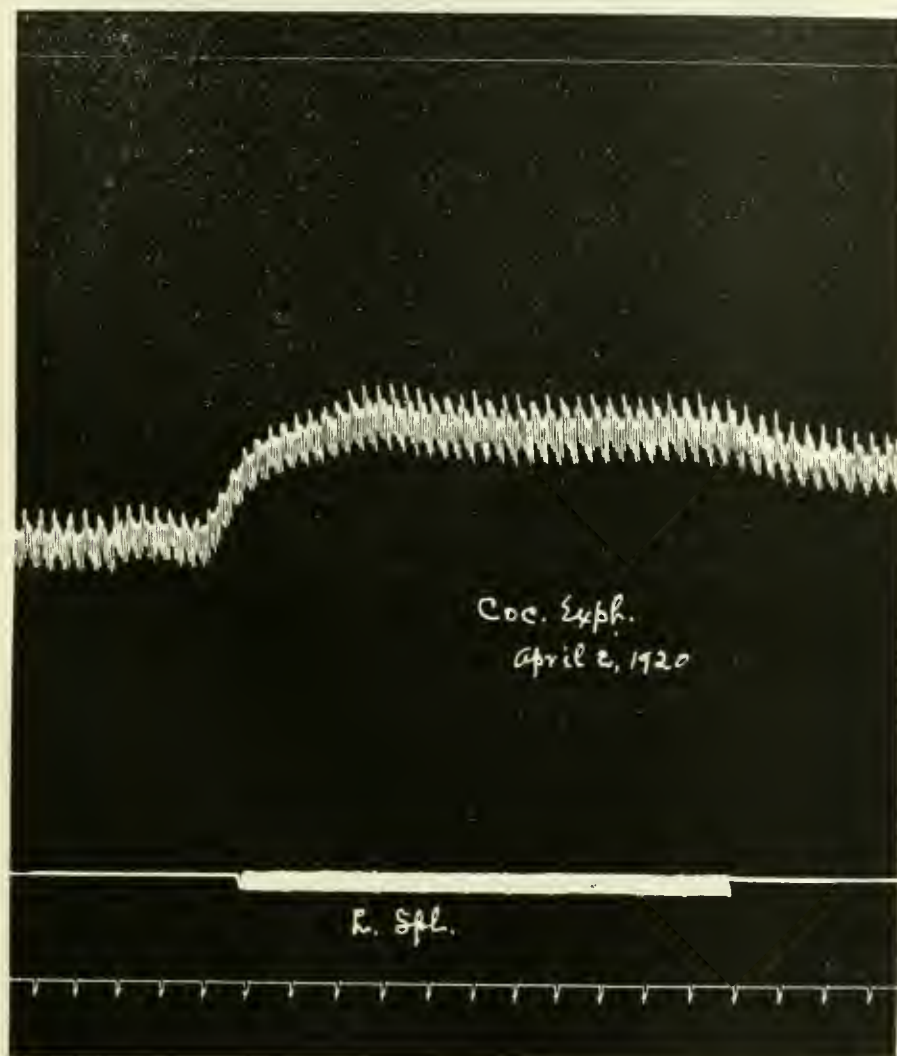
TRACING III

Dog. Ether anesthesia. Double adrenalectomy. Double vagotomy. Splanchnic nerve stimulation. Control.

of pressure change is typical of splanchnic stimulation with the preliminary rise, the "hump" followed by a deep notch or "dip" (Elliott (9), v. Anrep (10), Pearlman and Vincent (11)) then by a high and prolonged rise continuing as long as the current was

applied. To check up this point it seemed necessary to remove both adrenal glands prior to stimulation of the splanchnic nerve.

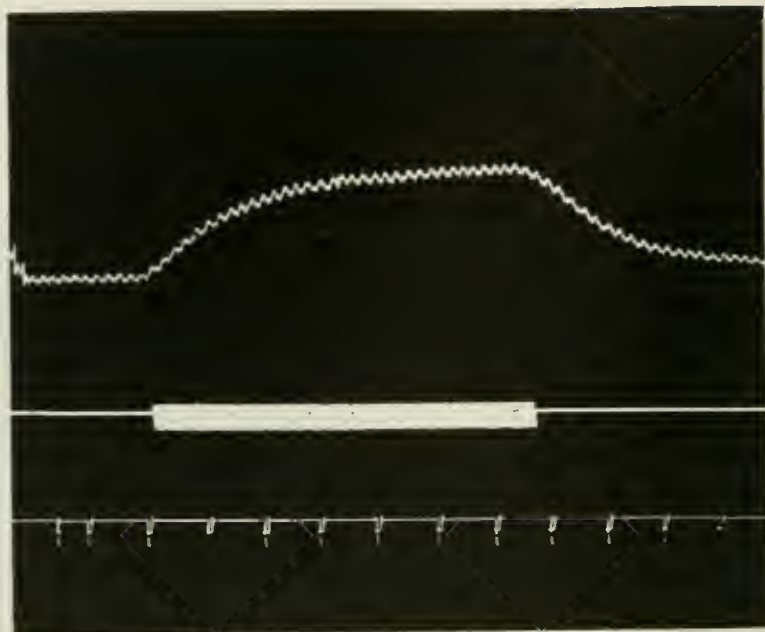
Curves of pressure changes are now found to be simple with a single rise with an increased pressure maintained throughout the



TRACING IV

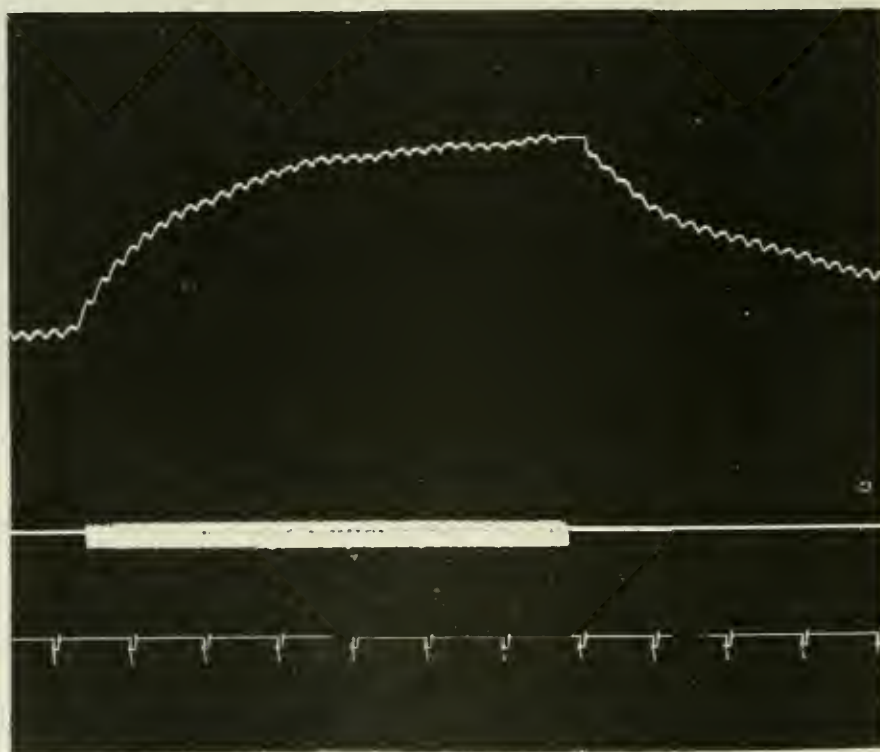
Same, after femoral vein injection of 5 cc. of 0.3 per cent solution of cocaine hydrochloride.

period of stimulation. Even here the response is found to be greater after cocaine than before. This eliminates the intermediation of the adrenal glands. Finally to eliminate changes in depth of anesthesia which result from cocaine administration,



TRACING V

Rabbit. Urethane anesthesia. Blood pressure record. Double adrenalectomy. Double vagotomy. Splanchnic nerve stimulation. Control.



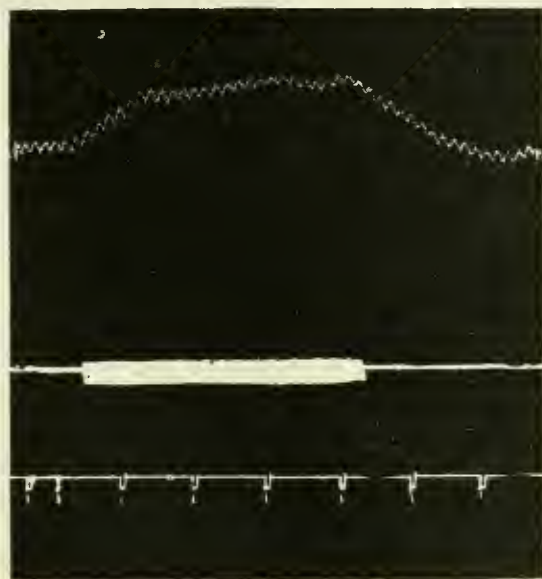
TRACING VI

Same after ear vein injection of 1.5 cc. of 0.3 per cent cocaine hydrochloride.

rabbits were used after decerebration, division of the cord at about the region of the first thoracic nerves and division of both vagi.

As may be seen from the tracings an increased response still occurred in such animals in which had been done decerebration, double vagotomy, transection of the cord and double adrenalectomy.

From such a series of experiments the conclusion seems warranted that on this particular part of the sympathetic nervous system the administration of cocaine increases the effects of elec-

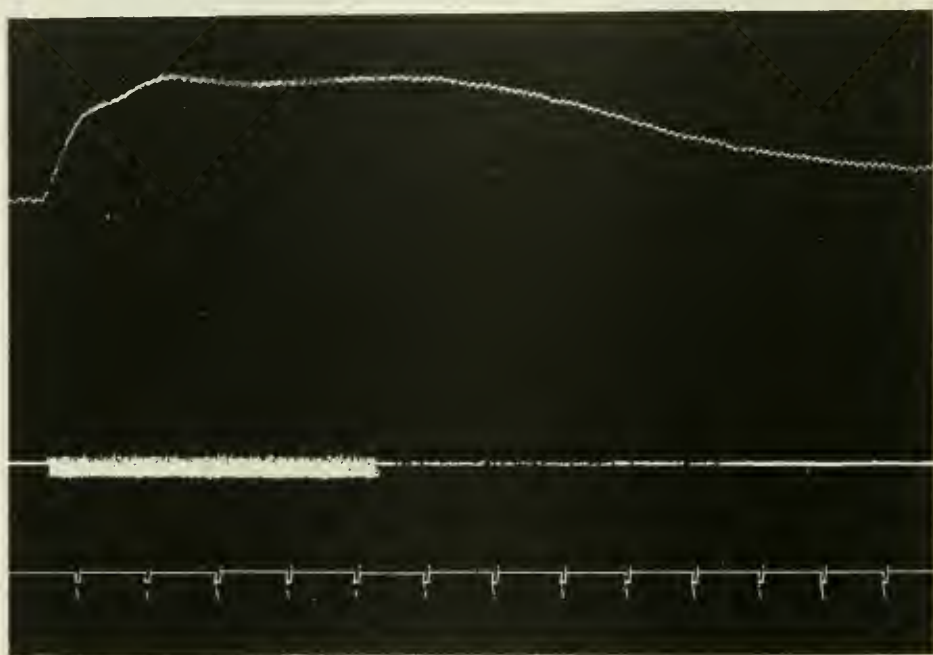


TRACING VII

Rabbit. Decerebration. Double vagotomy. Blood pressure. Splanchnic nerve stimulation. Control.

trical stimulation of the splanchnic nerve. The magnitude of the effects are much less owing to apparently simple reasons. After simple splanchnic stimulation with both adrenals intact the whole constricting mechanism of the body may be set into action by virtue of the outpouring of epinephrine which acting in conjunction (synergism) with cocaine similarly generally distributed produces a maximum response. On the other hand after double adrenalectomy, no epinephrine can be secreted by these glands hence splanchnic stimulation is of necessity directly

or indirectly limited to the endings of such nerves in the splanchnic area. Also, a considerable amount of damage is undoubtedly done to the sympathetic plexuses even by the most careful dissection, according to Stewart and Rogoff (12), Gley and Quinquoud (13) and others. Whether the nerve on one side communicates to sympathetic ganglia with fibers passing to all the abdominal viscera and tissues innervated by these splanchnic nerves is not a question involved in this paper.



TRACING VIII

Same after ear vein injection of 1.5 cc. of 0.3 per cent cocaine hydrochloride.

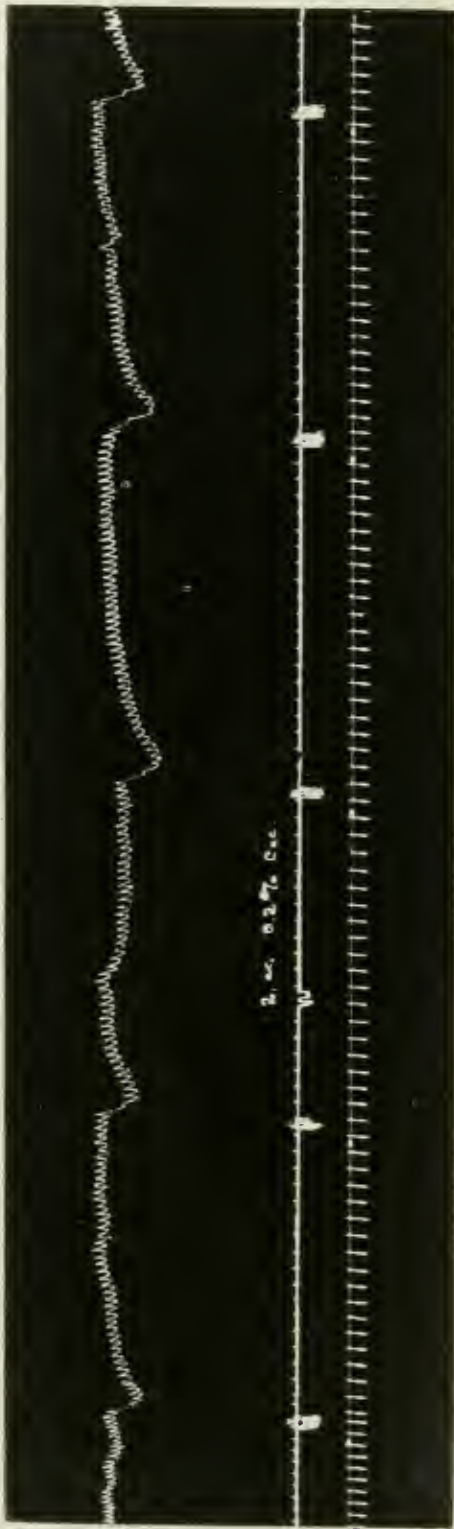
CERVICAL SYMPATHETIC NERVES

In order to apply the study to an available sympathetic nerve distant from and unconnected and uncomplicated by adrenal gland activity, the cervical sympathetic nerve was selected, using the nasal plethysmograph method of Tschalussow (14) as a test mechanism. This study was made on the dog and the rabbit in both of which it is relatively a simple matter to isolate the cervical sympathetic nerve. In the dog the nerve is readily found and separated from the vagus in the region of the superior sympathetic

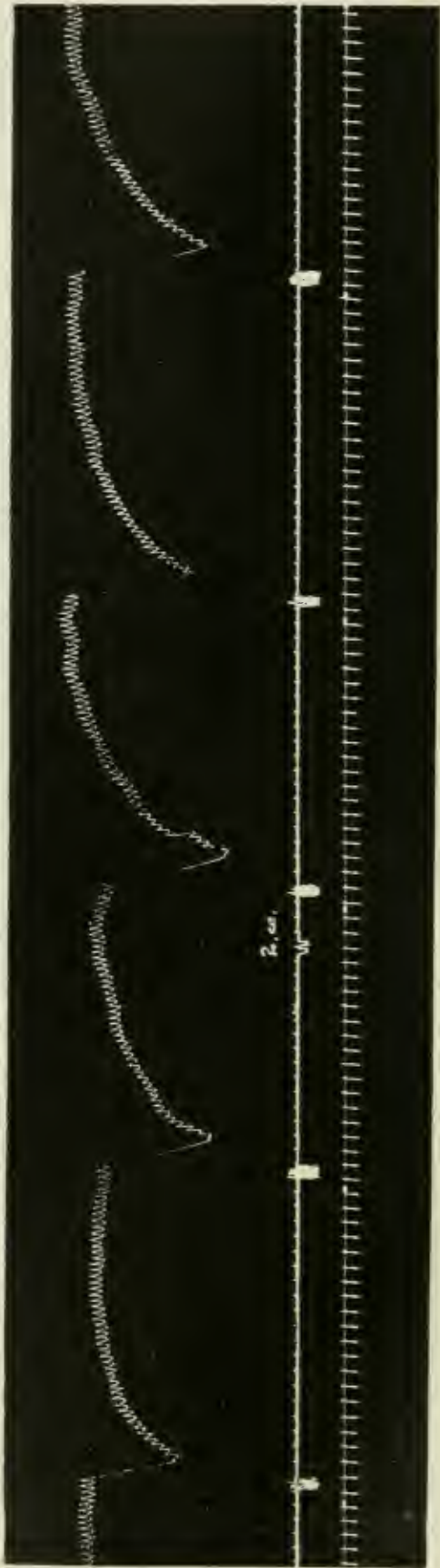
ganglion, at which point the sympathetic bundle may be readily separated for a convenient distance for purposes of application of electrodes. In the rabbit the sympathetic nerve lies separate throughout. The posterior nares were effectively closed by packing the throat with oiled cotton which also fills the mouth to form a rigid wall of the otherwise movable membrane over the cleft bony palate. The canine ducts were closed by thermocautery. A cannula was placed in one nostril, tied or more commonly packed securely in place by oiled cotton, the other nostril being solidly packed with oiled cotton. A good plethysmographic record can now be obtained. In the dog, it is possible to plug the posterior nares by oiled cotton plugs which require no further concern. Canine ducts and anterior nares were closed as in the rabbit. Best results were obtained only in such preparations as gave good pulse tracings. Strong healthy dogs were most satisfactory.

In regard to anesthesia it was soon found that, for this tissue at any rate, it is practically impossible to use either ether or urethane because of unavoidable changes in actual degree of etherization and depth of anesthesia since the slightest changes in ether concentration produce relatively enormous changes in blood flow to the nasal membranes. Furthermore since cocaine is a powerful central nervous system stimulant urethane is relatively uncertain since the depth of anesthesia is immediately changed on introduction of the drug. The only feasible procedure seemed to be to use an animal with cord transected and destroyed at the foramen magnum and destruction of a portion of the brain by inserting a probe or scalpel handle into the skull through this opening. With artificial respiration the preparation lasts for hours and is evidently entirely free from central nervous influences. Furthermore, the ether, which Mendenhall (15) has shown to be an especially poisonous anesthetic as regards to the threshold of sympathetic nerves, is entirely avoided after the short period required for decerebration.

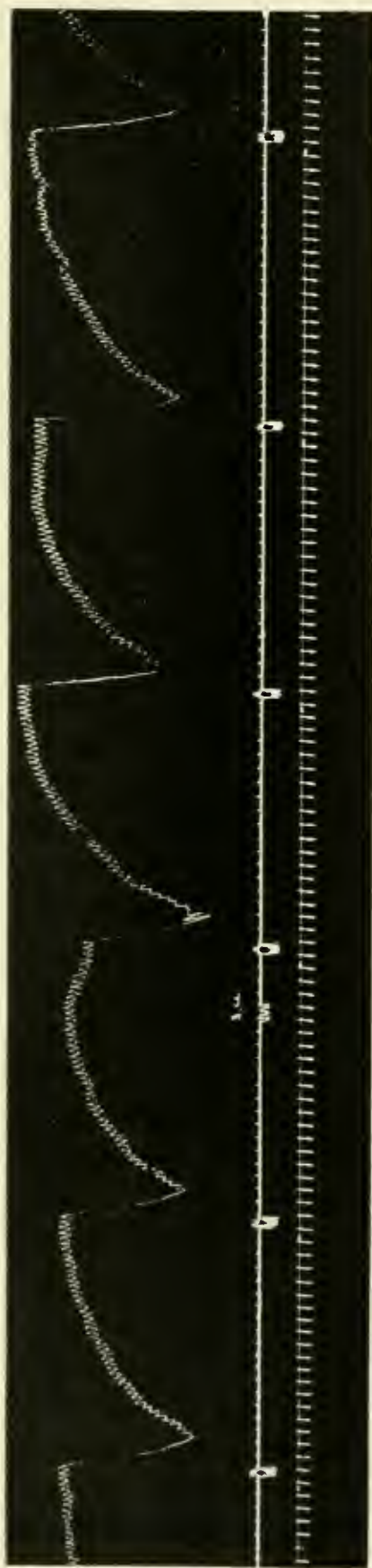
In this type of preparation it was found that cocaine actually increases the amount of response of the peripheral vasoconstrictor mechanism in the nasal chambers. It was also found that such



TRACING IX



TRACING X



TRACING XI

TRACINGS IX, X, XI. EFFECT OF STIMULATION OF THE CERVICAL SYMPATHETIC NERVE

Twelve kilogram dog. Brain and medulla pithed through the foramen magnum. Double vagotomy. Nasal plethysmogram. Stimulation at regular intervals and for the same duration controlled by clock-work. Time tracing, six seconds.

Before and after femoral vein injection of 2 cc. of 0.2 per cent solution of cocaine hydrochloride.

Curves of control and of cocaine effects are shown in each of the three tracings. Differences in amplitude in the three controls are due to differences in adjustment. Control and drug action curves were obtained under strictly identical and untouched adjustments.

small quantities as 0.2 mgm. cocaine in one cubic centimeter of salt solution injected into the femoral vein of a 12 to 15 kgm. dog produced a very marked nasal vasoconstriction which however is of short duration and often followed by a dilatation greater than before the drug was given. After a relatively short period the volume returns to normal again.

In two widely separated and unrelated systems of sympathetic nerves evidences are presented that cocaine actually renders the peripheral vasoconstrictor mechanism more irritable, as measured in amount of constriction produced by a short period of a near minimal electrical stimulus rather than a determination of the exact threshold limits as studied by Martin (16) and his associates.

Attempts to demonstrate a lowering of the threshold of the iris mechanism to electrical stimulation of the cervical sympathetic nerve have failed in our hands.

Thus we find that cocaine increases the responsivity of the peripheral neuromuscular mechanism to an adequate stimulus and that the so-called synergism between epinephrine and cocaine as regards vascular constriction is not one limited to the two drugs but that cocaine so affects the peripheral mechanism that such excitants as epinephrine and electrical stimulation both produce responses in excess of either alone without cocaine.

Since an actual lowering of the threshold of excitability has not been consistently found by the technique used, particularly in a diligent study of pupillary reactions, and since the responses to an effective stimulus were found to be greater after cocaine than before the conclusion is reached that the point of action of cocaine lies chiefly at some point or points beyond the fibers actually stimulated, namely, peripheral ganglia, myoneural junctions or muscle. While Niwa's results on isolated nerve preparations indicate an increased rate of carbon dioxide output after cocainization which may be interpreted as equivalent to an increased irritability of the fibers directly, our work so far has failed to confirm it when using vasoconstriction as a criterion. We take this to mean that our observed synergisms are largely if not completely due to changes in responsivity of some more peripheral component of the whole neuromuscular mechanism.

SUMMARY

1. Cocaine intravenously administered greatly intensifies the blood pressure rise after stimulation of the peripheral end of a divided splanchnic nerve.

2. This greatly increased response is largely due to the synergism between cocaine and the epinephrine secretion subsequent to splanchnic nerve stimulation.

3. In the absence of adrenal glands cocaine still increases the effects of splanchnic stimulation.

4. The nasal plethysmograph method of Tschalusow was used for the study of cocaine effects on the cervical sympathetic neuromuscular mechanism. By this means it was found that cocaine increases the constrictor effects of electrical stimulation of the cervical sympathetic nerves.

5. Attempts to demonstrate a lowering of the threshold of excitability of sympathetic nerves, particularly those of the pupillary mechanism, were not successful.

6. Since cocaine increases the effects of epinephrine and also of electrical stimulation of such sympathetic neuromuscular mechanisms studied, these increased effects appear to be largely if not entirely due to an increased responsivity of the peripheral neuromuscular mechanism rather than direct changes in threshold of excitability of preganglionic nerve fibers.

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THE PART PLAYED BY THE LIVER IN THE REGULATION OF BLOOD VOLUME AND RED CORPUSCLE CONCENTRATION IN ACUTE PHYSIOLOGICAL CONDITIONS

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It has long been known that acute changes in the number of red corpuscles per unit volume of blood take place under certain physiological and pathological conditions. It has been assumed that a certain part of this increase in number of erythrocytes was due to loss of fluid from the blood, but it is only recently that this has been experimentally proven. Everyone is familiar with conditions such as the excessive diarrhoea of cholera, the acute lung oedema in cases of gas poisoning, the endothelial poisoning after histamine injection, etc., in which there is a pathological loss of fluid from the circulation, but it is not with such conditions that we are here dealing.

Physiologically a variation in the number of red corpuscles per unit volume of blood occurs in many conditions: mental excitement such as fear and rage, exercise, asphyxia of any kind, and the injection of a substance which is a normal constituent of the body, namely epinephrin. The action of this substance on the concentration of erythrocytes in the blood has been carefully studied by the present author as a type of physiological polycythaemia which could be experimentally controlled, in order to ascertain if possible the location and type of process causing these changes (1). It was found that the liver is the seat of these changes. Epinephrin given intravenously caused a sudden marked increase in the red count, but after the removal of the liver from the circulation, the injection caused no change in the number of erythrocytes per unit volume of blood. Furthermore, animals whose liver does not respond to the action

of epinephrin, such as the rabbit, show no change in the red count after the injection of this substance. As epinephrin acted on the entire body with the exception of the liver, it was concluded that this organ was responsible for the change in red count.

The question next arose as to the manner in which the liver brought about this change in number of erythrocytes. Certain experiments were carried out by the present author which showed that there was no increased formation of red corpuscles, and that the blood volume was decreased. Other experiments were begun in 1916 to determine the action of epinephrin on the liver volume and lymph flow, but had to be postponed. In the meantime these experiments were reported by other investigators working along entirely different lines. Bainbridge and Trevan (2) found that after the injection of epinephrin there is a sudden enormous increase in the volume of the liver, which had been observed by other investigators, but they were able to account for this increase by finding an obstruction to the venous outflow from the liver, with a great increase in the portal pressure, but no change in the cava pressure. A complete explanation of the cause of this obstruction was not given, although they felt that it might be due to a swelling of the liver cells. That fluid actually escaped from the blood was shown by them and others (3), by the fact that there was a great increase in flow of liver lymph. These authors were not interested in the red count, but this loss of fluid actually demonstrated, will account for the increase in erythrocytes found by the present author.

Further evidence that fluid is actually lost in the liver and thus concentrates the blood and increases the red count is found in the effect of epinephrin after ligation of the hepatic artery. It was found as previously reported, that if epinephrin is injected after first ligating the hepatic artery, no increase in number of erythrocytes takes place. Bainbridge and Trevan found however that there is as great an increase in portal pressure and swelling of the liver if epinephrin is injected when the hepatic artery is tied, as when it is open. And Starling (3) had previously shown that ligation of the hepatic artery had no influence on the lymph

flow following the injection of epinephrin. If however fluid is lost in large amounts from the circulation and there is no change in the red count, red cells must have left the circulation in equal amounts. These cells cannot pass through the vessel walls, and therefore must be stored in the capillaries. And as this process takes place in the liver they must be stored in this organ. This was shown by the present author by first clamping the hepatic artery and then injecting epinephrin, as can be seen in the following experiment.

Experiment 163. Dog. 6.5 kilo. Ether

10.53	Reds, 7,960,000.
10.55	Clamped hepatic artery.
11.23	Reds, 7,696,000.
11.37	Reds, 7,976,000.
11.42	Clamp removed.
11.57	Reds, 7,816,000.
12.14	Reds, 7,600,000.
12.16	Hepatic artery again clamped.
12.19	Epinephrin 0.9 mgm. per kilo intravenously.
12.37	Reds, 7,704,000.
12.56	Reds, 7,224,000.
12.57	Clamp removed from artery.
1.15	Reds, 9,000,000.
1.27	Reds, 8,624,000.

The liver swells as shown by Bainbridge and Trevan, and there is an increased flow of lymph from the liver lymphatics, yet there is no change in number of erythrocytes per unit volume of blood. If however sometime after the injection the clamp is removed from the hepatic artery, and arterial blood again supplied to the liver, these red cells are immediately washed out into the circulation with as great an increase in the red count as if a fresh dose of epinephrin had been injected into the animal.

It has then been shown that after the injection of epinephrin fluid is lost from the liver, concentrating the blood and thus increasing the number of red cells per unit volume of blood. The question next arises as to whether other factors enter into this

increase in number of erythrocytes, which can only be determined by considering all possible ways in which an increase in number of erythrocytes per unit volume of blood might take place. Such an increase might occur by:

- (1) Loss of fluid with a concentration of the blood.
- (2) The new production of red cells.
- (3) A decreased destruction of erythrocytes in relation to production.
- (4) A redistribution of red corpuscles. i.e., a reservoir of red cells.
- (5) A division of erythrocytes.

As previously reported, there is no proof of an increased production of erythrocytes, of a decreased rate of destruction, or a division of red cells. The fact that an increase of one million or more in number of erythrocytes per cubic millimeter of blood takes place in from five to ten minutes, disappears in about an hour, and may be repeated many times in the same day, is against the production of new red cells, a decreased destruction, or the division of erythrocytes. We are then left with only two possible ways by which the number of erythrocytes may be increased, namely, loss of fluid from the blood, and a redistribution of corpuscles lying dormant somewhere in the body in greater concentration than in the circulating blood.

As regards the occurrence of a reservoir of erythrocytes, there is no actual proof of such. Schneider and Havens (4) have held the view that there is such a reservoir in the "splanchnic area," from which the erythrocytes could be brought out by massage. Massage of the liver alone causes no increase in the number of erythrocytes, and it has been observed by the present author that when the abdomen is opened and extensive operations done, that an increase in the red count takes place, but this is probably due to nerve stimulation rather than to massage. One condition has however been demonstrated in which the liver may act as a reservoir for red corpuscles and that is after the injection of epinephrin after ligation of the hepatic artery described above. As opening the hepatic artery washes these cells out into the circulation it may be supposed that in normal conditions where

the hepatic artery is open there will be very little sedimentation of corpuscles in the liver. We have then no proof that such a reservoir exists, and until one is experimentally demonstrated it seems most reasonable to account for the increase in red cells by fluid loss from the liver, which has been experimentally shown. From various experiments it would seem that when certain parts of the circulation are shut off by vasoconstriction as after giving epinephrin to a rabbit, that fluid and corpuscles are shut off in corresponding amounts as there is no change in the red count. On the other hand when fluid passes through the vessel walls, red cells are left in the capillaries in greater concentration. This is the case after the injection of histamine, and Cannon found an increase in the capillary blood in "shock," (5) in which it has been shown that there is a general loss of fluid into the tissues. And finally in the liver where the hepatic artery is tied, there is loss of fluid and a concentration of red cells in the capillaries. In physiological conditions no such fluid loss and obstruction to blood flow seems to occur except in the liver, and there, on account of the double blood supply, the corpuscles which ordinarily would be sedimented in the liver capillaries are washed on by the arterial blood stream.

In the one condition here analyzed, namely the injection of epinephrin, we have found that there is loss of fluid from the blood, and we have been unable to show that the red count is increased by any other means. The question next arises as to what variations in blood volume normally occur under physiological conditions, and where and how these changes are brought about.

Unfortunately our present blood volume methods are inadequate to differentiate between loss of circulating blood, as for example in vaso-constriction, and actual loss of fluid from the circulation as after the injection of epinephrin, where fluid is lost into the liver lymphatics. Our blood volume methods determine the volume of circulating blood, and not the actual blood volume as pointed out by Lamson and Nagayama (6). It is then necessary to study blood volume changes by indirect methods.

As shown above, after the injection of epinephrin, fluid escapes into the liver lymphatics, causing a concentration of blood. The question then arises as to whether fluid escapes from other parts of the circulation. There is a general belief that in physiological conditions fluid may escape from the circulation into the tissues generally, especially into the muscles. Such a view is held by Scott (7) who attributed the increase in number of red cells after the injection of epinephrin entirely to the forcing of fluid into the tissue spaces by the increased blood pressure, and from further experiments, concluded that any increase in the blood pressure forced fluid out of the circulation and caused an increase in the red count, while any decrease in pressure caused an inflow of fluid with a diminution in the red cell concentration. This view cannot be held by the present author because in animals in which the liver has been removed, or in which the hepatic artery has been tied, or in the rabbit whose liver does not respond to the action of epinephrin, the injection of this substance causes no increase in the number of erythrocytes per unit volume of blood although the pressure is enormously increased.

The work of Starling (3) on the lymph flow gives one an entirely different conception of the variation in the fluid content of the blood. Starling found that increasing the blood pressure by the injection of epinephrin, increasing the blood volume by the injection of saline, or by the injection of hypertonic salt or sugar solutions, which also increased the blood volume, caused a great increase in the flow of lymph from the thoracic duct, but practically no flow from the limbs. In fact he was unable to increase the flow of lymph from the limbs except by massage. He found that the lymph of the thoracic duct was composed chiefly of lymph from the liver, and the intestines, but that the increased flow following the above mentioned procedures was caused almost entirely by an increased flow of lymph from the liver, because if the liver lymphatics were first tied, no increase in the amount of lymph in the thoracic duct took place.

The fact that the injection of epinephrin causes a loss of fluid through the liver lymphatics with a concentration of the blood, which does not take place after the removal of this organ, to-

gether with the observation of Starling, that the only demonstrable changes in lymph flow under physiological conditions is from the liver lymphatics, points to the liver as the organ which is capable of regulating the blood volume under physiological conditions. It is an organ especially suited for this purpose, being the largest in the body, having the most permeable lymphatics, and the greatest lymphatic system with a direct communication via the thoracic duct to the venous circulation, and from its peculiar position in the body may slow the return of blood to the heart as after the injection of epinephrin, or may act as a reservoir for blood in an overdilated heart. By its size, its distensible capsule, and its free position in the body, it may take on enormous proportions without greatly interfering with the other organs, and tide over a time of stress by removing fluid from the circulation and returning it gradually through the lymphatic system.

An increase in the number of erythrocytes per unit volume of blood and consequently a decrease in the plasma volume has been found in many physiological conditions, exercise, all forms of asphyxia, and conditions of emotional stress. Cannon (8) has also shown that there is an increased output of epinephrin in all these conditions, and epinephrin has been shown to increase the red count. How great a part the secretion of epinephrin plays in the variation of the blood volume and red count has not yet been determined, but there is considerable evidence to show that nervous influences play a part, although all of these might equally well stimulate the adrenals. The fact that in conditions of emotional excitement the increase in red count is much more sudden, and far greater than after the injection of maximum doses of epinephrin is of interest but inconclusive as it may be due to setting free epinephrin under physiological conditions where the action is always greater, or to direct nervous influences. This matter still needs further investigation.

DISCUSSION

The polycythaemia in one acute physiological condition, namely that produced by the intravenous injection of epinephrin has been explained as stated above by the epinephrin causing an obstruction to the venous outflow from the liver, the passage of fluid out of the blood into the liver lymphatics, the washing on into the general circulation by the arterial blood stream of the red cells which cannot pass out with the plasma, and finally after the action of this substance passes off, the return of this fluid by way of the thoracic duct to the general circulation causing a fall of the erythrocyte count to normal.

We have seen that of all possible ways in which the red cell concentration of the blood might theoretically be increased there are only two which can occur in acute physiological conditions. One of these is by loss of fluid, and the other from the presence of a reservoir of red blood corpuscles in the body. No evidence of a reservoir of erythrocytes anywhere in the body unless it be in the liver has been demonstrated. The liver has however been made artificially to act as a reservoir of these cells by tying the hepatic artery and then injecting epinephrin, in which case fluid passes into the liver lymphatics and the red corpuscles are held in the small vessels, from which they can later be washed out by opening the hepatic artery. As clamping and unclamping the hepatic artery alone causes no change in concentration of erythrocytes, the liver does not normally act in this way as a reservoir for these cells. There is therefore under normal conditions no reservoir of red corpuscles in the body of sufficient magnitude to influence appreciably the concentration of these cells per unit volume of circulating blood. Acute physiological changes in the concentration of erythrocytes must then be due to plasma volume changes only. The plasma volume might be varied by loss of fluid through the lungs, gut, and kidney, or into the tissues generally. In one condition of maximum high blood pressure, namely after the injection of epinephrin, sufficient fluid is not lost from the blood to increase the number of red blood corpuscles appreciably if the liver is removed from the circulation.

Therefore simple pressure changes due to vaso-constriction will not cause fluid loss except through the liver. Acute polycythaemia has been found to occur in asphyxia of any type, exercise to a certain extent, emotional conditions of excitement, and after the injection of epinephrin. In each of these conditions epinephrin is supposed to be set free, and there is a condition of increased blood pressure, so that the polycythaemia could be accounted for as being of the epinephrin type. There are however so many possible ways by which fluid could leave the circulation, as through osmotic changes, excretion or secretion, that one cannot assume that all cases of acute polycythaemia take place in this one way. The liver however plays a much more important part in blood volume changes than has hitherto been supposed.¹

CONCLUSIONS

1. The sudden increase in number of red cells per unit volume of blood following the intravenous injection of epinephrin has been shown to take place in the following manner: an obstruction to the venous outflow from the liver, an increase in the portal pressure, passage of fluid into the liver lymphatics causing a swelling of the liver and concentration of the blood, followed by a gradual return of this fluid by way of the thoracic duct to the general circulation with a subsequent return of the erythrocyte count to normal.

¹ Physiologically the erythrocytes found in the general circulation are supposed to be mature, and not to undergo further division, no proof that they do divide has ever been given as far as the author knows. One finds however that there are conditions in which there is a sudden increase in number of erythrocytes with an increase in haemoglobin which is not proportional to the increase in number of erythrocytes. This fact is so often reported that in spite of the rather rough haemoglobin determinations made in most instances it is probably a true observation. The only way in which the number of erythrocytes per unit volume of blood could increase out of proportion to the haemoglobin would be by loss of haemoglobin from the corpuscles, the bringing into the circulation of corpuscles in which there is only a small amount of haemoglobin, or the division of erythrocytes. Experiments are being carried out by the present author in an attempt to account for the non proportional increase in red cells and haemoglobin in certain cases, which will be reported later.

2. The increase in number of erythrocytes per unit volume of blood after the intravenous injection of epinephrin does not take place from a general filtration of fluid into the tissues as supposed by some investigators, as no increase in the red count takes place in conditions of maximum pressure after removal of the liver.

3. Evidence is given to show that there is no reservoir of red corpuscles in the body normally, of sufficient magnitude to influence the red count appreciably.

4. All conditions of acute polycythaemia in which there has not been sufficient time for red cell production are due to concentration of the blood by fluid loss. The physiological polycythaemias of exercise, asphyxia, increased blood pressure, and emotional disturbances, could all be of the epinephrin type, namely temporary loss of fluid from the circulation through the liver lymphatics, although it is not yet proven that they are.

5. Two other types of acute polycythaemia occur, namely, those in which there is endothelial poisoning as after the injection of histamine, where fluid is lost into the tissues directly, and in cases of local irritation where fluid is secreted, as in the diarrhoea of cholera, and gas poisoning, in which enormous amounts of fluid are secreted into the bronchi.

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THE ACTION OF BORAX ON THE UTERUS

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Borax had a considerable reputation as an emmenagogue and ecboic in former days, and was on this account sometimes called "Sal uterinum." This action is still mentioned in many current text-books of pharmacology and toxicology. I have been unable to find in the literature any clinical evidence of this reputed action, or any experimental work to test its pharmacological basis. The following experiments were undertaken with this end in view.

EXPERIMENTAL

1. The action of borax on the excised uterus

The movements of the non-pregnant and pregnant uterus of the cat, rabbit and rat were recorded by Kehrer's method (1), in an apparatus similar to that described by Dale and Laidlaw (2). A horn of the uterus was suspended in oxygenated Locke's solution at 37°C. until the movements became regular, when measured amounts of a 1 per cent solution of borax were added and the effects recorded.

With concentrations of borax of 1 in 5000 and upwards in the fluid there was marked stimulation of the uterine movements, tone was increased and relaxation diminished. Figure 1 shows the effect of 1 in 5000 borax on the movements of the non-pregnant cat's uterus.

Borax is fairly strongly alkaline. The effect of alkalinity on the uterine movements was tested with sodium carbonate. It was found that 1 in 5000 sodium carbonate produced effects almost identical with those produced by the same strength of

borax. The effect of 1 in 4000 sodium carbonate on the uterus of the non-pregnant rabbit is shown in figure 3. Sodium carbonate in 1 per cent solution will neutralize about two and a half times as much normal acid solution as 1 per cent borax will do.

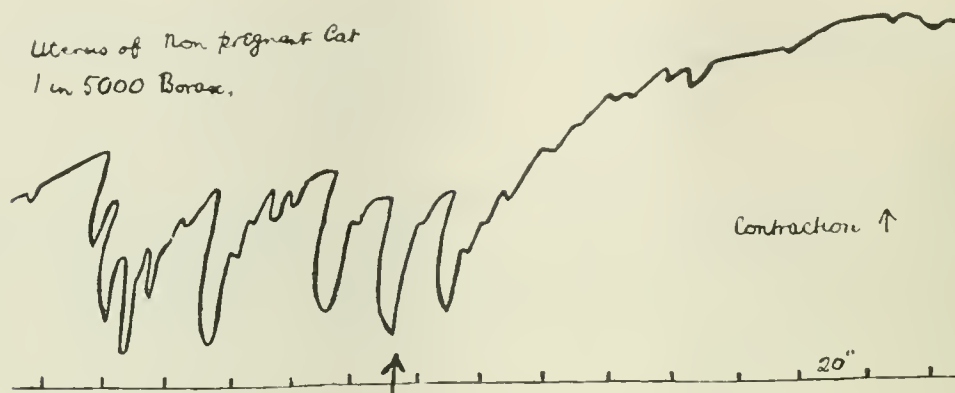


FIG. 1. SHOWING THE MOVEMENTS OF THE EXCISED UTERUS OF THE NON-PREGNANT CAT, AND THE EFFECT OF ADDING BORAX UP TO 1:5000



FIG. 2. SHOWING THE MOVEMENTS OF THE EXCISED UTERUS OF THE NON-PREGNANT RABBIT, AND THE EFFECT OF ADDING SODIUM CARBONATE TO 1:4000

To eliminate any action due to alkalinity a 1 per cent solution of borax was neutralized with a 1 per cent solution of boric acid. This solution had a much less powerful action on the uterus than borax, a concentration of 1 in 2000 of this combined neutral solute

being required to produce the same increase of movement as follows 1 in 5000 of alkaline borax. Figure 3 shows the action of 1 in 4000 neutralised borax on the movements of the uterus of the non-pregnant rabbit.

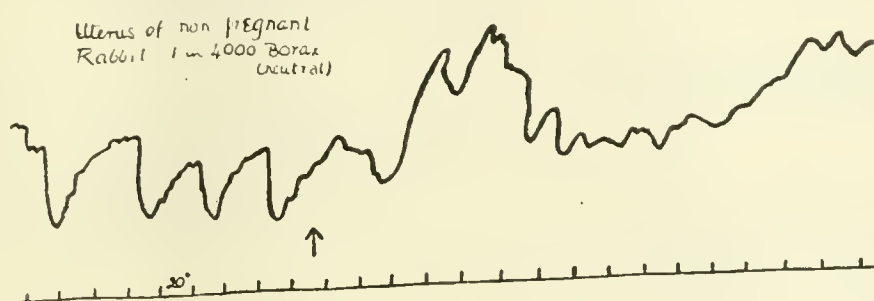


FIG. 3. SHOWING THE MOVEMENTS OF THE NON-PREGNANT UTERUS OF THE RABBIT, AND THE EFFECT OF ADDING NEUTRAL BORAX (SEE TEXT) UP TO 1:4000

Boric acid produced no effect until enough was added to alter the reaction of the medium, when the uterine movements were inhibited.

Borax apparently acts directly on the uterine muscle, for no difference was noticed in the reaction of the non-pregnant and the pregnant cat's uterus.

2. *The action of borax on the uterus in situ*

The action of borax on the surviving uterus was controlled by a series of experiments in which the movements of the uterus of the pregnant cat and rat were recorded in situ. The animal was anaesthetised, and placed in a bath of warm saline solution (85 per cent sodium chloride in tap water). The abdomen was opened, the two limbs of a Cushny myocardiograph (3) fastened to one of the horns of the uterus at a distance of 1 to 3 cm. apart, and the movements of the intervening portion recorded.

If large doses of borax were administered intravenously (in the cat, 40 mgm. per kilo of body weight), there was stimulation of movements, and increase of tone. The stimulation was not

great and did not last long. Figure 4 shows the stimulation that followed injection of 0.1 gram of borax into the jugular vein of a non-pregnant cat weighing 2150 grams.

In a few experiments injection of neutral solutions seemed to be followed by slight stimulation, but in the majority they produced no effect.



FIG. 4. SHOWING THE MOVEMENTS OF THE UTERUS OF A NON-PREGNANT CAT RECORDED IN SITU, AND THE EFFECT PRODUCED BY INTRAVENOUS INJECTION OF 0.1 GRAM BORAX

DISCUSSION

The experiments on the isolated uterus show that borax in large doses has a stimulating action on the uterus which is partly due to alkalinity. This was confirmed in the experiments on the uterus in situ. The action is feeble when compared to other uterine stimulants, for example, quinine, which produces an effect on the isolated uterus in concentrations of 1 in 100,000, and on the uterus of the cat in situ if an intravenous injection of 10 mgm. be given.

Borax has, however, apparently very slight toxicity. Binz (4) records a case in which 18.75 grams were taken within three hours without producing any other symptom than vomiting and diarrhoea. In such a case, provided the greater part of the drug were not lost owing to the gastro-intestinal effects enough might be absorbed to have an action on the uterus. The intestinal irritation would no doubt induce pelvic congestion and reflex contractions of the uterus, and this may perhaps explain the tradition that borax possesses an emmenagogue or ecboic effect.

From the experiments it seems unlikely that any dose likely to be given therapeutically can have any direct or indirect action on the uterus.

SUMMARY

1. Borax has a direct stimulant action on the uterus in concentrations corresponding to toxic doses.

2. This action is largely due to the alkalinity, as neutral solutions have less effect.

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THE INFLUENCE OF DIURESIS ON THE ELIMINATION OF UREA, CREATININE AND CHLORIDES

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The effect of changes in blood flow through the kidney on the secretion of various constituents of the urine has been examined recently by Marshall and Kolls (1). In these experiments the one kidney of a dog was compared with the other, the blood flow of one being increased by section of the corresponding splanchnic nerve or decreased by partial constriction of the renal artery. The elimination of water and chlorides is very markedly affected by changes of blood flow; that of urea is definitely affected but to a less extent than water and chlorides, while that of creatinine and phenolsulphonaphthalein is very slightly if at all influenced by this procedure. The increased elimination of urine by the kidney with the greater blood flow can be considered in the nature of a diuresis as compared to the secretion of the organ on the normal side. The similarity of the changes from increased blood flow to those characteristic of an increased flow of urine were discussed previously (2), but it was pointed out that sufficient accurate data were not available for a completely satisfactory comparison. The effect of diuresis on the elimination of urea, creatinine, and chlorides has not been examined simultaneously. The experiments reported here were carried out with the idea of determining if the same differences in the excretion of these substances was apparent during diuresis. Not being convinced at present that during all forms of increased urine flow produced by different diuretics the elimination of substances would be the same, the present investigation has been mainly concerned with the so-called water diuresis, or the increased flow of urine produced by the ingestion of water.

It is generally stated and accepted that the chloride of the urine more or less parallels in excretion the water. Likewise, the elimination of urea is known to be increased by the ingestion of large amounts of fluid, while the excretion of creatinine is independent of the urinary volume, and is but little effected by the ingestion of fluid. Most of the experiments on these points, however, have been carried out over twenty-four hour periods. It is quite probable that the extent of the changes present during the water diuresis may be masked or compensated for in the long periods of collection.

The drinking of large quantities of water causes some increase in the nitrogen and other constituents in the urine when measured over twenty-four hour periods. This has been ascribed to a "flushing out of the tissues" (diuresis), to an increased protein metabolism or to both. No attempt will be made to review the voluminous literature on this subject, but reference will be made to a few papers bearing somewhat on the question in point. The earlier literature can be found in an article by Hawk (3), who in a reëxamination of the question found the nitrogen, sulphur, and phosphorus increased in experiments on men in nitrogenous equilibrium. Heilner (4) gives a good review of the literature on the excretion of nitrogen, and believes that whether the nitrogen is increased or not depends on whether the animal is fed or starving. Fowler and Hawk (5) in one subject on fixed diet and régime found the addition of 3000 cc. of water per day to increase the total nitrogen, urea, and ammonia on the first day, to decrease the creatinine (2 per cent), and cause the excretion of creatine. Howe, Mattill and Hawk (6) in a fasting dog in the advanced stages, found the additional ingestion of water to increase the total nitrogen (77.5 per cent), urea, ammonia, creatinine (30 per cent) and cause the appearance of creatine in the urine on the first day of increased water intake. Wilson and Hawk (7) experimenting on two normal men on a uniform simple diet found with increased water intake (distilled water) an increase in ammonia, no change in the phosphate, and a decrease in the chloride elimination. Orr (8) experimenting on men found the excessive ingestion of water caused increased excretion of urinary nitrogen

which is more marked on a low protein diet. He found an increase in the per cent of nitrogen as urea, a marked increase in the excretion of ammonia, but no change in the creatinine and no excretion of creatine.

The data on the elimination of urea, creatinine, and chlorides during shorter periods of time and the effect of diuresis on this elimination are very scant and meagre. Numerous investigators have studied water diuresis, but few analysis of the dilute urines obtained as compared with the normal are to be found. Cushny in discussing the dilute urine obtained from drinking large quantities of water states,

In this dilute urine, the percentage of all the solids is much reduced, while the total solids and the absolute amount of urea, phosphate, sulphate and potassium excreted in a unit of time is increased. The total amount of chloride excreted during the diuresis, as compared with that normally in the urine, depends rather on the richness of the tissues in salt than on the extent of the diuresis. It increases somewhat in the dog and often in man (9).

Addis and Watanabe (10), in their recent investigations on the rate of urea excretion have concluded that in man there is no appreciable effect on the rate of urea excretion from changes in urine volume. Under certain conditions, when no food or water have been taken for some time, a large water diuresis causes a marked increase in the elimination of urea without any change in the concentration of urea in the blood. Addis, Barnett and Shevsky (11), likewise conclude that in the rabbit the volume of urine does not influence the rate of urea excretion. Van Hoo-genhuyze and Verploegh (12) and Klercker (13) studying problems of creatine and creatinine metabolism in man give figures for short periods on the elimination of creatinine. Their results indicate in general a constancy from hour to hour. The experiments of Shaffer (14) on the excretion of creatinine in man indicated a much more remarkable constancy in the excretion of this substance from hour to hour. He noted the independence of the excretion on urinary volume, output of total nitrogen, and in a few instances studied the effects of water diuresis and diure-

tin. The excretion was apparently independent of the diuresis. Neuwirth (15), in a recent paper, on the hourly elimination of certain urinary constituents during brief fasts working on one subject found very wide variations in the hourly excretion of creatinine, as well as total nitrogen and uric acid.

One point that appears extremely important in an investigation of the effect of diuretics or in fact of any procedure on the elimination of any urinary constituents is the performance of control experiments without this procedure under as nearly the same conditions as possible. This has been generally neglected in diuretic experiments, one or more control periods being taken before the diuretic was administered. The investigations of Addis and his co-workers (16) on the urea excretion in rabbits have shown that wide variations occur without any procedures other than catheterizing, bleeding, and the passage of the stomach tube. In animals, deprived of food and water for seventeen hours before the experiment, hourly determinations of the amount of urea excreted exhibited a progressive rise from hour to hour without any corresponding rise in the blood urea concentration. The increase frequently amounted to over 100 per cent. Again the experiments of Mendel and Staele (17) and Neuwirth (15) in which the hourly excretion of uric acid was measured in man show wide variations in the hourly output. Certain determination which I have carried out on man on the hourly excretion of phosphates showed marked variations from hour to hour, and some experiments on the hourly excretion of creatinine in the rabbit illustrate the same point. When a few preliminary experiments were carried out on the effect of water diuresis on the excretion of creatinine in the rabbit, a marked increase was observed during diuresis. One or more control periods were taken before the administration of the diuretic, but control experiments over the same period of time without the administration of any water frequently exhibited the same increases in the excretion of creatinine. Proper control experiments over the same periods of time and under the same conditions are essential, as these frequently show variations of such a magnitude that if a diuretic had been administered at a certain point, the change which

occurs in the control would be ascribed to the diuresis. Undoubtedly some of these variations are not variations in the activity of the kidney in excreting the substance in question, but variations in the production of the substance in the organism. These effects can be taken into account by a simultaneous analysis of the plasma, but the effects noted by Addis in regard to urea excretion in rabbits cannot be explained on the basis of a change in the blood.

METHODS

One series of experiments were carried out on normal men. A control experiment was performed in all cases without the ingestion of water, but otherwise under similar conditions as the diuresis experiment which was made the following day. Urine was collected in hourly periods, and the elimination of water, chlorides, urea, and creatinine determined. The earlier experiments were conducted after a simple breakfast. Considerable variations were encountered in the hourly excretion of urea and chlorides under these conditions, so in most of the experiments the subjects had no food after 7:00 p.m. the preceding evening. Water was withheld during the experiments except when given to produce diuresis as stated in the protocols.

The experiments on dogs were made without anaesthesia, and were similar to those upon the human subjects. The bladder was emptied by catheter, and washed with 20 cc. of warm sterile distilled water. Blood samples were drawn from the jugular or superficial leg vein with a needle and pipette.

The urine samples of the different periods of any one experiment were diluted to the same volume before analysis. Urea was determined by the author's urease method, creatinine by Folin's method (18), and chlorides by the Volhard or in the case of some of the animal experiments by the Van Slyke-McLean method (19). The plasma was analyzed for urea by the urease method, for creatinine by Folin's original method (20), and for chlorides by the Van Slyke-Donleavy modification of the Van Slyke-McLean method (21). The estimation of creatinine *in plasma* by the original Folin method has been shown to be fairly

accurate by numerous observers in the last few years (22). The interpretative curves as suggested by Hunter and Campbell (23) were used in the estimations of plasma creatinine.

RESULTS

The following experiments were carried out on one subject (E. K. M.) after a light breakfast at 9:00 a.m. which was identical in all the experiments. The diuresis experiment was performed the day following the control.

TABLE 1
Excreted per hour

TIME	URINE	CREATI- NINE	UREA	NaCl	TIME	URINE	CREATI- NINE	UREA	NaCl
	cc.	mgm.	mgm.	mgm.		cc.	mgm.	mgm.	mgm.
9:00-10:00	43	66	930		9:00-10:00	38	65	837	516
10:00-11:00	45	68	1221	588	10:00-11:00	90	66	1170	570
11:00-12:00	54	70	1166	760	11:00-12:00	715	62	1377	700
12:00- 1:00	43	63	975	626	12:00- 1:00	720	64	1251	521
1:00- 2:00	43	66	942	600	1:00- 2:00	240	65	893	400
					At 10:30-10:40 drank 1500 cc. water				
9:00-10:00	32	58	809	380	9:00-10:03	31	60	762	418
10:00-11:00	37	66	1038	427	10:03-11:01	32	66	943	402
11:00-12:00	51	65	1113	679	11:01-12:00	300	65	1420	822
12:00- 1:00	38	60	945	355	12:00- 1:00	665	61	1239	511
					1:00- 2:00	107	61	773	245
					At 10:50-11:03 drank 1200 cc. water.				

These two experiments demonstrate very clearly that water diuresis has little if any effect upon the excretion of creatinine. In the control of the first experiment, the creatinine varied from 63 to 70 mgm. During the diuresis experiment it varied from 62 to 66 mgm. With an excretion of 38 cc. of urine, there were 65 mgm. of creatinine, while with an excretion of 715 cc. there were 62 mgm. of creatinine. The variation in urea from hour to hour during the controls makes it a little difficult to interpret the diuresis results, but it appears evident that a decided increase in the excretion of urea is present in both experiments. The chlorides

appear to be definitely increased in one experiment and unchanged in the other.

The same type of experiment was performed on several subjects who had fasted from 7:00 p.m. the preceding evening. The actual times of collection of the urine samples are given, while

TABLE 2
Excreted per hour

TIME	URINE	CREATI- NINE	UREA	NaCl	TIME	URINE	CREATI- NINE	UREA	NaCl
Subject E. K. M.									
	cc.	mgm.	mgm.	mgm.		cc.	mgm.	mgm.	mgm.
8:45- 9:50	37	62	648	553	9:00-10:00	46	67	821	630
9:50-10:50	35	66	726	465	10:00-11:00	53	65	936	690
10:50-11:50	38	63	747	550	11:00-12:00	353	62	1109	702
11:50-12:50	39	64	687	555	12:00- 1:00	596	63	1069	528
12:50- 1:50	32	65	576	450	1:00- 2:00	210	62	738	312
					10:50-11:00 drank 1200 cc. water				
Subject S. G.									
8:55- 9:55	50	77	1158	705	9:00-10:00	42	73	1027	564
9:55-10:55	39	77	867	625	10:00-11:00	45	72	911	672
10:55-11:55	36	73	875	590	11:00-11:50	250	76	1440	804
11:55-12:55	21	64	540	240	11:50-12:50	605	70	1346	405
12:55- 1:55	18	68	505	210	12:50- 1:50	109	67	816	102
					10:50-11:00 drank 1250 cc. water				
Subject W. O.									
8:26-10:15	40	86	1074	462	7:59- 9:39	36	81	837	477
10:15-11:20	87	85	1236	576	9:39-10:41	121	88	1206	702
11:20-12:15	58	78	984	384	10:41-11:40	762	79	1281	431
12:15- 1:14	67	83	1062	396	11:40-12:40	595	86	1251	398
					10:10-10:18 drank 2000 cc. water				

the amounts of urine, urea, creatinine and chlorides are calculated for one hour periods.

In the experiments recorded in table 2, where the subjects fasted, the excretion of urea and chlorides were somewhat more regular than in those in table 1. Even here variations of some magnitude occur in some of the controls, and it is noticed that

the urea and chloride appear to follow the urine volume while the creatinine does not. The absence of any effect of diuresis on the excretion of creatinine is evident, while a decided increase in the elimination of urea occurs during the diuresis. An increase in the chloride elimination is also evident, although it may be very slight.

TABLE 3
Excreted per hour

TIME	URINE	CREATI- NINE	UREA	NaCl	TIME	URINE	CREATI- NINE	UREA	NaCl
Subject V. R. D.									
	cc.	mgm.	mgm.	mgm.		cc.	mgm.	mgm.	mgm.
1:00-1:58	32	75	520	390	1:00-2:00	44	65	672	530
1:58-3:02	30	69	523	350	2:00-3:00	85	66	888	660
3:02-3:59	18	65	390	172	3:00-4:00	488	55	834	490
3:59-5:00	12	67	360	130	4:00-5:00	515	66	933	330
					2:30-2:45 drank 1500 cc. water				
Subject S. W. F.									
1:00-2:00	28	67	519	455	1:04-2:00	39	64	712	590
2:00-3:05	22	64	450	388	2:00-3:00	60	67	867	730
3:05-4:01	15	70	334	225	3:00-4:00	490	65	990	450
					4:00-5:00	455	64	825	200
					2:30-2:45 drank 1500 cc. water				
Subject G. K. D.									
(1:00-1:58	17	31	260	115)	1:00-2:00	97	57	444	518
1:58-3:02	38	64	660	220	2:00-3:00	330	59	549	638
3:02-3:59	36	65	540	200	3:00-4:00	720	59	540	398
3:59-5:00	25	61	455	178	4:00-5:00	670	61	489	255
					2:30-2:45 drank 2000 cc. water				

The following experiments were made in the afternoon on subjects who had the same breakfast on two successive mornings, but omitted the midday meal (table 3).

The results are similar to those previously recorded. The creatinine is not affected by the increased flow of urine, urea is definitely increased, and chlorides are slightly increased.

Table 4 indicates the results of experiments on dogs. The collections of urine in these experiments were made for one-half hour

periods. These short periods of collection, however, did not show near as great a constancy in the excretion of creatinine as longer ones of an hour or hour and a half. The results are tabulated in terms of one hour or one and a half hour periods and here show a remarkable constancy in the creatinine excretion. A distinct increase in the elimination of urea during diuresis is apparent, while no effect is apparent on the elimination of creatinine.

It is quite possible to argue that in the experiments which have been discussed the kidney has not exhibited a difference in the behavior to creatinine, urea and chloride during diuresis. One

TABLE 4

TIME	URINE	CREATININE	UREA
<i>Experiment 1.</i> Dog. D1. Collie, female, weight 18.2 kilos. No food for previous twenty-four hours. At 11:14 given 500 cc. water by stomach tube.			
	cc.	mgm.	mgm.
9:41-11:11	14.2	33.6	928
11:11-12:41	75.0	33.0	1368
12:41- 2:11	164.5	31.1	1012
<i>Experiment 2.</i> Dog D2. Female, weight 11.8 kilos. No food for previous twenty-four hours. At 11:47, 500 cc. of water by stomach tube.			
10:17-11:17	6.1	10.8	399
11:17-12:17	6.8	11.0	423
12:17- 1:17	63.5	12.1	702
1:17- 2:17	170.5	11.9	590
2:17- 3:17	175.5	11.5	541

might assume that the concentrations of these substances in the blood have been so changed as to affect the elimination during diuresis. However, the investigations of Engel and Scharl (24), Macallum and Benson (25), and Haldane and Priestley (26) have shown that the dilution of the blood after drinking large quantities of water is too small to detect by changes in the refractive index of the serum, red count or haemoglobin estimations. Priestley (27) detected a slight fall (2 per cent) in the electrical conductivity of the serum after water drinking, and attributed this to the passage of salts from the blood to the intestine. Goldschmidt and Dayton (28) studying absorption from

the colon, found that the chloride content of the plasma might fall due to the passage of chlorides from the blood into water placed in the large intestine. It appears impossible then to explain the changes as due to changes in the plasma. Table 5

TABLE 5
Excreted per hour

TIME	URINE	CREATI- NINE	UREA	NaCl	TIME	URINE	CREATI- NINE	UREA	NaCl
Dog, female, weight 7.7 kilos									
	cc.	mgm.	mgm.	mgm.		cc.	mgm.	mgm	mgm.
9:40-10:56	2.4	9.2	153	6.0	9:17-10:20	2.1	9.0	162	7.9
10:56-11:41	2.7	10.1	148	10.6	10:20-11:21	2.3	9.7	180	15.6
11:41-12:42	2.2	9.2	159	9.1	11:21-12:26	16.6	8.3	267	7.0
12:42- 1:40	2.4	8.9	152	8.3	12:26- 1:23	38.2	10.0	294	12.0
					At 10:55 given 350 cc. water				
10:58 Plasma	Creatinine	0.90			10:35 Plasma	Creatinine	0.88		
	Urea	23.0				Urea	25.0		
	NaCl	693.0				NaCl	674.0		
12:50 Plasma	Creatinine	0.91			12:35 Plasma	Creatinine	0.94		
	Urea	26.0				Urea	24.0		
	NaCl	705.0				NaCl	655.0		
Dog, female, weight 15.7 kilos									
10:02-11:01	1.5	19.5	153	31.0	9:25-11:03	3.5	20.6	180	13.2
11:01-12:00	3.6	21.8	180	72.1	11:03-11:48	8.6	23.2	348	26.0
12:00-12:56	5.2	22.7	204	74.0	11:48-12:45	198.0	22.1	354	28.3
12:56- 2:02	3.6	20.2	192	71.0	12:45- 2:29	26.8	20.7	285	16.2
					At 11:20 given 500 cc. water				
11:07 Plasma	Creatinine	1.14			11:07 Plasma	Creatinine	1.12		
	Urea	21.0				Urea	26.0		
	NaCl	700.0				NaCl	692.0		
1:00 Plasma	Creatinine	1.18			12:48 Plasma	Creatinine	1.10		
	Urea	22.0				Urea	23.0		
	NaCl	690.0				NaCl	670.0		

contains experiments on dogs with plasma analyses before and during the diuresis. The control experiments were carried out the day preceding the diuretic experiments under the same conditions except no water was given during the experiment. The

figures for urea, creatinine, and chlorides in plasma are expressed in milligrams per 100 cc.

The results confirm the idea that changes in the plasma cannot be responsible for the changes in the urine. In the first experiment, the creatinine excretion shows no change during diuresis, while the urea is increased over 80 per cent at the height of the diuresis. The concentrations of urea and creatinine in the plasma have apparently undergone little change. The chloride in the urine is not increased appreciably, but since a distinct fall of the chloride of the blood has occurred, the activity of the kidney in eliminating chloride has been increased. The second experiment exhibits similar findings. In diuresis produced in dogs by the intravenous injection of hypertonic sodium chloride, sodium sulphate or urea solutions, the creatinine was not increased or indeed changed in a few experiments which have been performed. The creatinine elimination is not influenced by many other diuretics than water.

DISCUSSION

The results which have been obtained indicate definitely the same difference in the behavior of urea and creatinine during water diuresis as is observed with changes in blood flow through the kidney. Urea is increased to a variable extent in different experiments, but the increase may amount to 80 per cent or more. An increase in the creatinine cannot be detected because if present it is much smaller than the variations which occur in control experiments. The excretion of chloride from hour to hour is so irregular under the conditions of the experiments that the amount of increase during diuresis cannot be determined. Frequently, however, a very definite increase is shown especially in the first period of the diuresis and before it is at its height. The increase in chloride is certainly not greater or as striking as the increase in urea.

The greatest increases in urea and chloride do not always and in fact seldom occur in the same period as the greatest increases in water elimination. This fact has been already commented upon by Addis (11) in regard to urea in his experiments where

water was given after abstention from food and water. He concluded that the increase urine flow was not responsible for the increase excretion of urea, but that both might be due to some common cause. This behavior of urea is similar to that found by Van Slyke, Stillman and Austin (29) in that an increase in the urinary volume accelerates the excretion of urea up to a certain point (5 liters in twenty-four hours). When the excretion of chloride is increased it is during the earlier stages of the diuresis. At the height of the urine flow, the elimination of chloride is diminished.

The "modern theory" of the secretion of urine as outlined by Cushny (30) in his monograph requires that during "dilution diuresis" all the "no-threshold" substances, e.g., urea, phosphates, sulphates, etc., should be increased, and, moreover, since these substances are not reabsorbed the percentage increase should be the same in all these substances, unless the composition of the plasma has markedly changed. The marked increase in urea during water diuresis, and the absence of a demonstrable increase in creatinine under the same condition is rather opposed to this view. The experiments of Marshall and Kolls (1) on changes in blood flow affecting appreciably the elimination of urea, and not that of creatinine are along the same line. Again, the concentration of "no-threshold" substances by the kidney should be the same according to the "modern theory." The figures in this paper indicate that in the same sample of urine the dog's kidney may concentrate creatinine very much more efficiently than urea. For instance, in the first period of the experiment in table 5 creatinine is concentrated about 425 times, and urea only about 280 times. Creatinine is apparently a "no-threshold" substance, as injections into dogs with frequent analysis of plasma and urine indicate that the rate of excretion is roughly proportional to the concentration in the plasma. The behavior of various other non-threshold substances will have to be investigated and more data accumulated before it can be determined whether some modification of the "modern theory" may explain the facts. This question will be more fully discussed in a later communication.

SUMMARY

The effect of the ingestion of large quantities of water (water diuresis) on the elimination of creatinine, urea and chloride has been studied in normal men and dogs. The urine volume is frequently increased twenty-fold or more; creatinine is not increased to a measureable extent; urea is increased definitely but never more than two-fold; chlorides are apparently increased but the increase is variable and generally less marked than that of urea. The increases in urea and chloride do not correspond with the maximum increase in water excretion, in fact at the height of the diuresis the chloride elimination generally decreases. During water diuresis the chloride of the plasma may decrease, while the concentrations of urea and creatinine in the plasma do not vary appreciably.

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THE INFLUENCE OF SACCHARIN ON THE CATALASES OF THE BLOOD

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Some of the recent work on saccharin has had a tendency to ascribe to this substance an action beneficial to the processes of oxidation in the body, particularly in diabetes. For this reason it was considered worth while to subject the action of this drug to further study with particular reference to its effect upon the catalytic power of the blood.

The first investigators we observed to have studied the influence of saccharin upon the catalases of the blood were Mathews and McGuigan (1). Using an exceedingly simple method they found that 0.25 gram of saccharin intravenously reduced the power of the blood to release oxygen from hydrogen peroxide. This effect apparently was only a temporary one. They showed also that saccharin added to blood *in vitro* produced the same results.

More recently Burge (2), working on dogs, has made the assertion that the introduction of 4 grams of saccharin per kilo of body weight into the stomach of a normal dog will produce an enormous increase in the catalytic power of the blood of the animal. In the single experiment given in his paper the increase amounted to 10 per cent in the first hour, 54 per cent in the first two hours, and 56 per cent in the first two and one-half hours. The same writer also claims (3) that saccharin increases the catalytic power of the blood in diabetic dogs to a remarkable degree. In the three experiments given the increases were 42, 44, and 49 per cent during the first hour after the administration of the drug into the stomach and bowels, after which no further figures are given.

A few observations regarding these experiments must be made: Since it has already been shown (4) that the error in this type of experiment is great, we must know the number of determinations made on each animal and the variation between these determinations. It is not clear from the data given whether the animals were or were not under the influence of an anaesthetic—this is relatively unimportant from our own experience but very important from the experience of Burge (5). No statement is made regarding the actual amount of gas liberated by the blood of the animal before or after the action of saccharin. Since only percentage of increase is given, it is not clear whether a 40 per cent increase involved the release of 4 cc. more gas—all that would be necessary if the dog released 10 cc. normally, or 40 cc. of gas—the volume necessary if the dog yielded 100 cc. before the action of the drug began. We would be adverse to considering an increase of 4 cc. of gas to be of much if any significance because of the inaccuracies of the method, unless the small increase were consistent in repeated tests on the same animal, and the same increase appeared in a number of animals. On the other hand an increase of 40 cc. of gas would be considered significant without question for it is outside the range of errors so common to this type of experiment. Yet where percentages only are given, as is the case in the papers under discussion, an increase of 4 cc. produces just as marked a rise in the curve as a rise of 40 cc. in another animal.

Recently Stehle (6) published data which do not agree with Burge's results on the effect of foods and other substances—among them saccharin—on the catalytic power of the blood, and concludes: "fluctuations in catalase content are due to fluctuations in the number of red cells in the blood. . . . It is simpler to regard catalase content as a function of the number of red cells than to assume a direct relation between catalase and biological oxidations."

Recognizing the inaccuracies of the method, and with the knowledge of what significance would be read into the data by those interested in the substitution of saccharin for sugar, it was considered desirable to make a further study of the action of saccharin upon the catalases.

MATERIALS AND METHODS EMPLOYED

The saccharin used in this work was purchased on the open market and was labelled sodium benzoysulphonicimide, or "Sweetening Powder." It was manufactured by the Heyden Manufacturing Company of Garfield, New Jersey. It was a fine white powder, soluble in 1.56 parts of water, intensely sweet and then bitter to the taste, and yielded a neutral solution when dissolved.

The hydrogen peroxide employed was the ordinary 3 per cent preparation, acid in reaction and preserved with acetanilid. Most of the work was done on sample I, a preparation which required 7.5 cc. of $\frac{N}{2}$ NaOH to neutralize 1000 cc. This sample was kept during the period of experimentation, June 1 to September 15, in the cooling room of the morgue just above the freezing point. Sample II was manufactured by the same firm as sample I but required 15 cc. $\frac{N}{2}$ NaOH to neutralize 1000 cc. It was kept under the same conditions as sample I. All the peroxide was warmed to room temperature and neutralized just before using. So far as we are able to judge the two samples of peroxide under the conditions of the experiments would yield the same amount of oxygen with equal amounts of the same blood. Dog 6 was the only animal besides one human subject with experiments on both samples. These samples were studied chemically by Dr. C. S. Smith and were found to comply with the U. S. P. requirements. Not quite one-half of the oxygen in the peroxide solution was released by an experiment with human blood.

The method employed in the experiments was the one described in an earlier paper (4). It consisted of adding measured amounts of blood to 50 cc. of hydrogen peroxide in a Bunsen bottle and collecting the gas in a large burette above distilled water. During the evolution of gas—which was permitted to continue at room temperature for ten minutes—the bottle was shaken at a uniform rate by means of a motor. Five separate bottles and burettes were employed for each determination, thus the figures given will be—unless otherwise stated—the average of five determinations corrected for temperature and pressure.

In the case of dog's blood, 5 cc. of the thoroughly defibrinated blood was diluted with an equal volume of 0.9 per cent salt solution. Of this mixture 1 cc. was added to each bottle. Thus the figures given are the amounts of gas released from 50 cc. of hydrogen peroxide by 0.5 cc. of dog's blood.

In the case of cat's blood, 1 cc. of the defibrinated blood was diluted with 9 cc. of 0.9 per cent salt solution. Of this mixture 1 cc. was added to each bottle. Thus the figures given are the amounts of gas released from 50 cc. of hydrogen peroxide by 0.1 cc. of cat's blood.

In the case of human blood—where the catalytic power is still greater—the dilutions were 1 cc. diluted with 39 cc. of 0.9 per cent salt solution. Thus the figures given show the amounts of gas released by 0.025 cc. of human blood from 50 cc. of hydrogen peroxide.

CATS

In the experiments on the action of saccharin on the catalytic power of the blood it early became evident that the controls would be fully as important as the action of the drug itself. For that reason it was considered advisable to study the effect of this drug upon the catalytic power of the blood of cats as well as upon the blood of dogs. Knowing that the blood of the former shows a decidedly greater catalytic power than that of the latter, it was considered quite possible that the amount of deviation from the normal might be correspondingly greater under the influence of the drug. Table 1 shows the effect upon the catalases of approximately 4 grams of saccharin per kilo dissolved in water injected into the lower end of the oesophagus which had been ligated above the level of the injection to prevent the possibility of regurgitation. The animal was kept under light ether anaesthesia during the whole experiment, and bled from the jugular every fifteen minutes for the first hour, and at the end of two and of three hours. The column marked before shows the amount of oxygen released immediately after surgical anaesthesia had been established. As controls, cats were anaesthetized and given like amounts of water in the same manner

without saccharin, kept under ether and bled at the same intervals. In table 1 can be seen the results of five experiments and three controls.

The determinations on test animals were thirty-five in number. It must be remembered that the figure given is the average of five tests on the same blood and peroxide under identical conditions—five were made on blood drawn before, thirty after the administration of saccharin. Study of the table shows that of

TABLE 1
Effect of saccharin on cats

NUMBER	WEIGHT	SACCHA- RIN	WATER	GRAMS PER KILO	BEFORE	AFTER (MINUTES)					
						15	30	45	60	120	180
	<i>grams</i>	<i>grams</i>	<i>cc.</i>								
1	3395	8.5	35	2.5	17.8	18.8	21.0	19.5	17.8	18.5	16.5
2	2335	7.0	25	3.0	26.7	22.3	21.4	21.9	23.6	23.2	19.5
3	2875	14.0	35	4.8	24.0	26.1	23.3	24.4	25.1	25.2	23.1
4	3140	8.0	20	2.6	64.4	63.2	62.0	63.7	73.7	69.8	61.3
5	2895	10.0	25	3.5	38.5	32.6	37.9	35.2	32.2	35.5	32.8
Average.....					34.28	32.60	33.12	32.94	34.48	34.44	30.64

Controls											
1	1730	0	30	0	28.3	28.3	24.2	23.8	26.3	23.7	20.1
2	1950	0	30	0	27.9	26.1	24.0	24.4	24.0	22.6	24.4
3	4070	0	30	0	57.2	49.6	48.7	52.6	49.8	50.0	50.7
Average.....					37.80	37.10	32.30	33.60	33.36	32.10	31.73

This table shows the effect of the injection into the oesophagus of cats of from 2.5 to 4.8 grams per kilo of body weight of soluble saccharin upon the catalytic power of cat's blood.

the 30 determinations made after saccharin, nineteen showed a slight decrease, ten a slight increase, and one no change whatever. This study shows, also, that there is no definite regularity regarding the time at which the increases come, in fact no increase was observed in cats 2 and 5. There was a slight increase in two cases (1 cc. and 2.1 cc.) after fifteen minutes, in one case (3.2 cc.) after thirty minutes, in two cases (1.7 cc. and 0.4 cc.) after forty-five minutes, in two cases (1.1 cc. and 9.3 cc.) after

sixty minutes, in three cases (0.7 cc., 1.3 cc. and 5.4 cc.) after two hours and in no case after three hours. It was pointed out in our earlier article that there is considerable error in the method, for that reason the only increases mentioned which we consider important are those of 9.3 cc. and 5.4 cc. which are not appre-

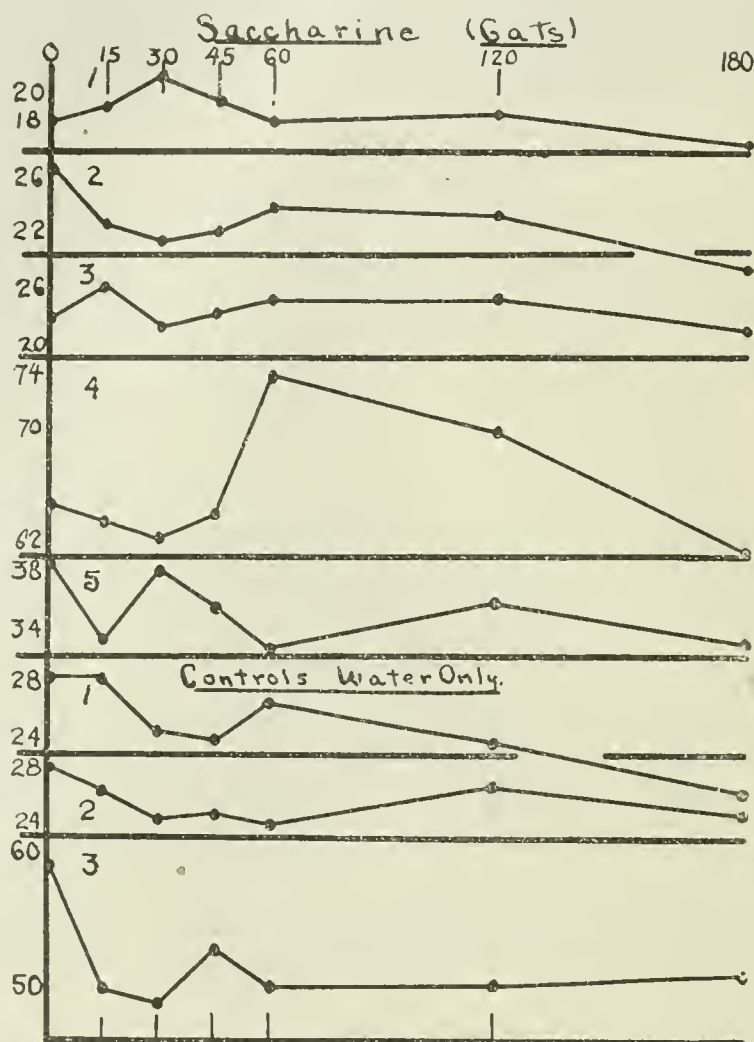


FIG. 1. EFFECT OF SACCHARIN ON THE CATALASE CONTENT OF THE BLOOD OF CATS

ciably greater than changes in the opposite direction. The average of all tests taken at any definite period after the administration of saccharin showed a decrease—slight—after 15, 30, 45, and 180 minutes, and an increase—very slight—after one hour and after two hours—in these cases 0.2 and 0.16 respectively.

In fact the changes in the catalase content of the blood of cats under the influence of saccharin were so slight as to be negligible, many of them being due to experimental error.

The controls show also a slight fall under the normal reading. See figure 1 which gives graphically the variations in catalase content of all the test animals and the controls, also figure 12 which shows the averages of the test cats and the controls.

RESULTS ON DOGS

So long as animals were to be under observation for long periods of time it was considered advisable to study the variations in catalase content of the blood under laboratory conditions in order to be sure regarding normal fluctuations. The blood was tested frequently, often twice a day even if the animal was not used for an experiment. In tables 2 and 3 we give the data secured from dogs observed under identical conditions of housing, food, and water. It is evident from the figures and tables that the catalase content of the blood even under good laboratory conditions varies within wide limits. Also these variations are enormously greater in animals with high catalase content as in dog 5, than in animals with low catalase content as in dog 1 (see table 5 and 9, figures 4 and 6).

In table 2, we give the data secured from two dogs. Experiment days are marked clearly both in the table and in figure 2 which shows graphically the data recorded in table 2. It is evident that the catalytic power varies between wide limits. In dog 5 the high point was 91.6 cc. released on the 79th day of observation. The record shows that this high point came when the dog was in an advanced stage of pancreatic diabetes. The low point was 51.1 cc. This point was observed at a time when the animal was in excellent physical condition. In dog 6 the high point was 71.7 on the fifty-first day, in the afternoon of the day of removal of practically all the pancreas. The high point thus came on a day after the animal had been deprived of food and water for twenty-four hours preparatory to the operation. At this time the blood was undoubtedly concen-

TABLE 2
Variations in the catalytic power of blood of dogs

DOG 5			DOG 6		
Day	Oxygen released		Day	Oxygen released	
	A.M.	P.M.		A.M.	P.M.
1	84.5	86.9	1	40.5	
2	58.0	85.2	2	33.3	
3	80.6	84.3	3†	29.7	27.2
4	80.9	67.9	4	27.0	
5*	80.7	73.6	5	25.0	
6	71.0	86.6	6§	20.2	21.3
8	69.5	67.8	8	20.8	
9†	66.3	64.4	36	62.9	
10	72.5		43	57.6	
12		64.2	44*	62.0	57.5
13	71.5		45	54.1	
15*	65.2	66.6	46*	64.2	57.3
16	59.1		47	46.6	
18	75.3		48	50.7	
19‡	75.2	76.3	50**	50.9	64.6
22	75.9		51	71.7	66.4
23	76.7		52	60.4	57.4
24‡	81.8	82.4	53	51.0	
25	72.4		58‡	45.1	45.1
26	69.4		59	41.2	
27§	64.5	63.2	69‡‡	53.4	
29	70.3		73‡	36.7	37.5
57	75.1		80‡	46.5	46.1
64	76.3		81	24.2	
65*	65.5	65.2	84	46.7	
69	61.4		85‡	45.9	46.6
70*	64.8	60.7	86	41.1	
72	64.6		87§	43.7	41.5
73	73.9		89	44.5	
74**	74.3	72.2	92	40.8	
75	80.4		93*	38.6	42.9
79*	91.6	84.7			

* Saccharin by mouth.

† Bled only (control).

‡ Saccharin intravenously.

§ NaCl intravenously (control).

** Pancreas removed.

‡‡ Second pancreas operation to remove vestiges on seventieth day.

This table shows the variation in the catalase content of dogs observed over long periods. The figures are cubic centimeters of oxygen released from 50 cc. hydrogen peroxid in ten minutes by 0.5 cc. of dog's blood.

trated by restricted fluid intake. The graph shows a sharp decline when the animal was given water *ad lib.* after the operation. The low point, 20.2 cc., appeared on the sixth day, a time when the animal was in such poor physical condition that her survival was in doubt. A rest of twenty-six days, however, resulted in a perfect recovery. During this period the catalytic power of the blood increased markedly.

Comparison of dogs 5 and 6 shows that the low point may be coincident with marked impairment of the physical condition as in dog 6, but such need not be the case as is shown by dog 5. Neither does the high point fall with the best physical condition

TABLE 3

Daily variation in catalase content of blood of experiment animals

NUMBER	SEX	WEIGHT	DAY OF OBSERVATION																	
			1		2		3		4		5		6		7	8		9		10
			A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.		A.M.	P.M.	A.M.	P.M.	A.M.
1	F	13	10.3	12.9	13.0	17.0	12.6	12.0	9.2		11.9	10.9	8.1	7.9		16.7		23.0	15.4	18.2
2	M	10	86.5	65.0	64.3	70.2	62.8	60.4	53.4		56.5	54.0	52.7	53.1		55.0	49.7	44.5		43.2
3	M	12	43.2	38.9	33.2	35.4	39.0	36.9	28.9		34.9	32.6	33.8	35.6		37.9	32.3	36.8		40.4
4	M	11	40.2	36.3	36.1	35.1	36.1	34.4	31.8	33.1	33.6		29.6	32.8		27.1		22.1	20.6	23.7
5	F	15	84.5	86.9	58.0	85.2	80.6	89.3	80.9	67.9	80.7		71.0	86.6		69.5	67.8	66.3		72.5

for in both cases the high point came, in 6 after removal of the pancreas, in 5 after the animal had been anaesthetized for the final experiment when the animal was certainly in an impaired physical state.

In our opinion the variations in any one animal are due entirely to the alteration of the number of corpuscles, alteration due largely to water intake and elimination. Repeated experiments with the unavoidable bleedings tend also to decrease the number of red cells per unit of volume, and hence the catalase content. We can see every reason for the belief that we can vary at will the catalase by altering the water content of the blood. This view is supported by Stehle, who found that hydrogogue cathartics (MgSO_4) produce a parallel increase in catalytic power and

data on the diurnal variations must now be presented. These experiments were performed on normal dogs by drawing blood at 9.00 a.m. and again at 2.00 p.m., thus after a five-hour interval. There were some minor variations but this was the average schedule. The data is presented in table 3 and figure 3, and

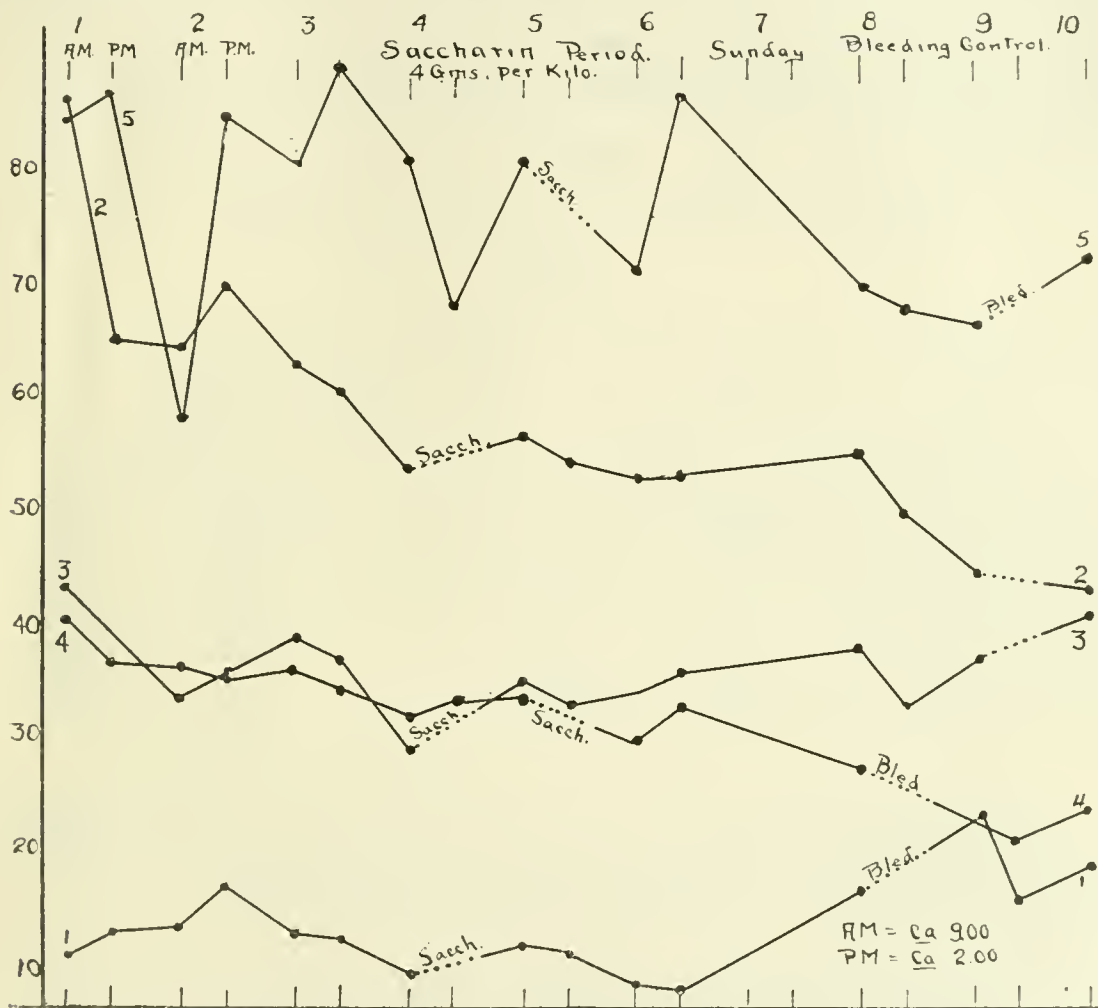


FIG. 3. DAILY VARIATIONS IN THE CATALASE CONTENT OF THE BLOOD OF DOGS

a summary in table 4. As can be seen from table 3 the data was secured from five animals each of which was subjected to two experiments, one with saccharin, one with bleeding during the period of observation. The experiment days are indicated clearly, only the morning data is recorded, and that data from the experiments performed on these dogs can be seen in tables 5 and 9 and figures 4 and 6. As can be seen best from the sum-

mary in table 4 there was a total of thirty days of observation. On twelve days the catalase content rose between the first and the second bleedings; on eighteen days it fell during the same time. The catalase content rose thus normally in 40 per cent of all cases and fell in 60 per cent. The greatest rise in volume was 27.2 cc. or 46.8 per cent above the morning observation in dog 5. The greatest fall in volume was 21.5 cc. in dog 2. The greatest fall on the percentage basis was 33 per cent in dog 1, where the actual volume of gas involved was only 7.6 cc. This fact shows clearly the error of curves plotted on the percentage

TABLE 4
Summary of table 3

DOG	RISES			FALLS		
	Number	Greatest rise		Number	Greatest fall	
		Cubic centi- meters	Per cent*		Cubic centi- meters	Per cent*
1	2	4.0	30.7	4	7.6	33.0
2	2	5.9	9.2	4	21.5	24.8
3	2	2.2	6.6	4	5.6	14.7
4	2	3.2	10.8	4	3.9	9.9
5	4	27.2	46.8	2	13.0	16.0
	12			18		

* Morning reading was taken as the starting point because all tests were begun in the morning and thus a morning reading was used as a normal uniformly throughout the work for experiment as well as for control dogs.

rather than on the volume basis; for a fall of 7.6 cc. in the case of dog 1 gave a percentage decrease of 33 per cent, while a fall of 21.5 cc. in the case of dog 2 gave a percentage decrease of only 24.8 per cent. Thus we may expect between the hours of 9.00 a.m. and 2.00 p.m. a fall in 60 per cent of cases and a rise in 40 per cent of cases of animals without drugs. The greatest rise we observed in the normal animal by volume was 27.2 cc. or 46.8 per cent of the morning observation; the greatest fall was 21.5 cc. or 33 per cent. We are now prepared to study the action of bleeding upon the catalase content of the blood.

In table 5 and figure 4 we show the data from animals bled exactly as animals were bled for saccharin experiments: They were bled first for a normal, then six other samples were taken after 15, 30, 45, 60, 120 and 300 minutes. Since one of these samples, the first, constitutes the normal, and since one sample of blood in this series was lost by imperfect defibrination in the case of dog 4 we have 29 determinations to compare with the normals. As can be seen best in table 6, dogs bled in the manner

TABLE 5
Influence of bleeding on catalase content of the blood

DATE	NUMBER	SEX	WEIGHT	SACCHARIN			BEFORE		AFTER							
				Amount	Grams per kilo	NaCl	24 hrs.	5 min.	15 min.	30 min.	45 min.	1 hr.	2 hrs.	5 hrs.	24 hrs.	
6-9	1	F	13	0	0	0	7.9*	16.7	15.2	24.1	18.4	18.9	23.9	19.7	23.0	
6-9	2	M	10	0	0	0	55.0	44.5	47.9	45.4	45.6	47.3	37.8	41.8	43.2	
6-10	3	M	10	0	0	0	37.9	36.8	30.9	33.3	32.0	28.7	32.7	32.2	40.4	
6-9	4	M	11	0	0	0	29.6*	27.1	26.4	†	24.9	32.1	25.4	24.3	22.1	
6-10	5	F	15	0	0	0	69.5	66.3	69.2	69.6	63.2	61.9	59.8	64.4	72.5	
Average							39.98	38.28	37.92	34.48	36.82	37.78	35.92	36.48	40.24	

* First reading forty-eight hours before test.
† Blood lost.

described show a rise in 13 of 29 cases, or in 44.8 per cent; and a fall in 16 of 29 cases, or in 55.1 per cent. The greatest rise was in dog 1, 7.4 cc. or 44.3 per cent in thirty minutes; the greatest fall was in dog 3, 8.1 cc. or 22.0 per cent in one hour. The data here is not materially different from that secured from dogs bled twice per day as can be seen by comparison with table 4. In table 7 and figure 5 we give the data from dogs bled as above, but which receive varying amounts of warm 0.9 per cent salt solution immediately after the first bleeding. These tests control the effects of the salt solution in which the saccharin was dissolved in the intravenous experiments with that drug. In table 8 we give the summary of the results. It can readily be seen from this table that there were rises normally in 7 of 18

cases—38.8 per cent and falls in 11 of 18 cases—61.1 per cent. The greatest rise in volume was 1.5 cc. in dog 5, the largest percentage rise was 5.4 per cent in dog 6. The greatest fall was 11.2 cc.—27.7 per cent in dog 3.

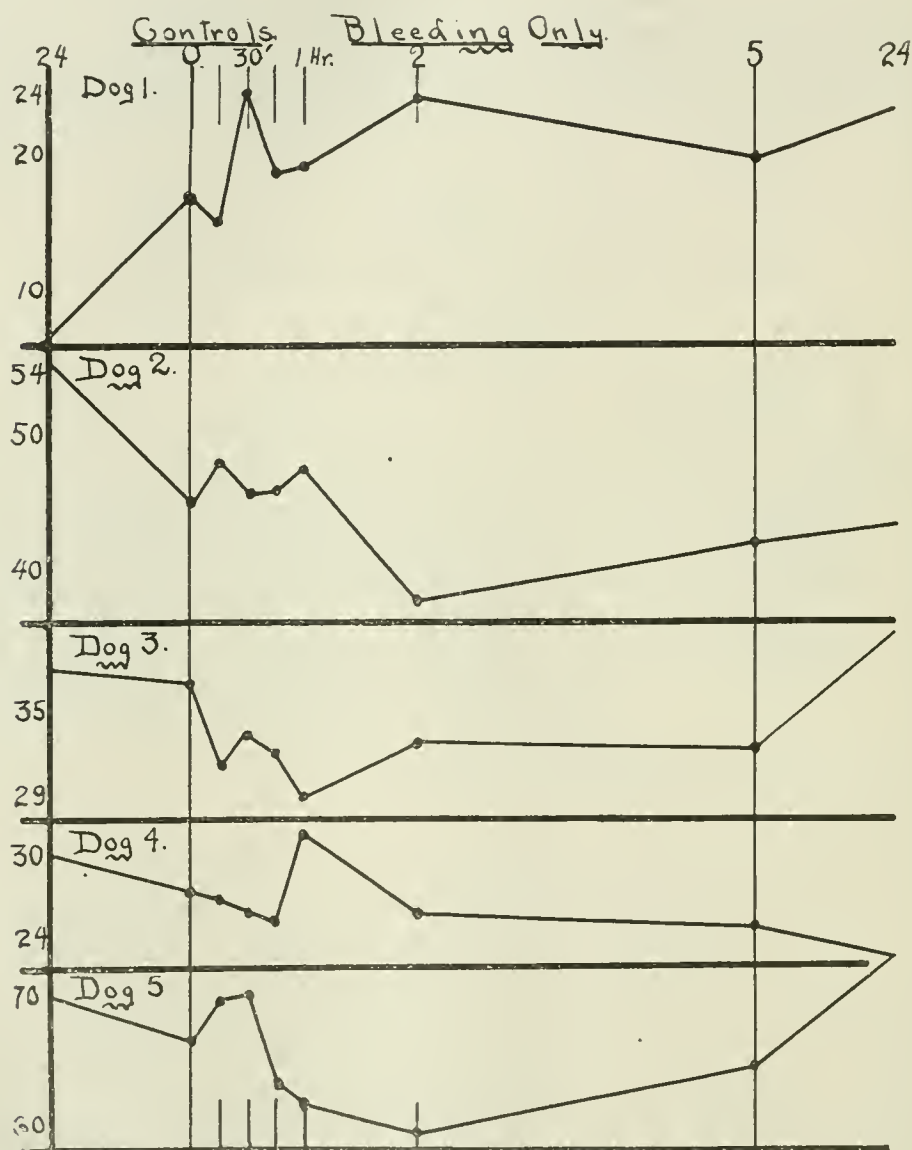


FIG. 4. EFFECT OF BLEEDING ON THE CATALASES OF THE BLOOD

Having established clearly by the data in tables 2 to 8 and figures 3 to 5 variations for which we must make allowances in considering the effect of drugs because of normal variation, the effect of bleeding, and of the injection of salt solution, we are prepared to study the effect of saccharin. As has already been

stated Burge (2) (3) finds a marked increase in the catalytic activity of the blood of normal and diabetic dogs following the administration of saccharin by mouth in doses of 4 grams per kilo. Stehle (6) finds an increase in 3 cases of 5 studied. The increases amounted to 14, 15, and 25 per cent respectively of the first or normal reading. In the remaining 2 cases no increase was noted. The dose used was 5 grams per kilo. His record shows fluctuations between wide limits in some cases; e.g., in experiment 1 he records 215 cc. at 60 minutes after the drug; 233 cc. at 95 minutes; 215 cc. at 145 minutes; and 233 at 160

TABLE 6
Summary of table 5

EXPERI- MENT	NORMAL	RISES				FALLS			
		Number	Greatest rise			Number	Greatest fall		
			Cubic centi- meters	Per cent	Time		Cubic centi- meters	Per cent	Time
1	16.7	5	7.4	44.3	30 min.	1	1.5	8.9	15 min.
2	44.5	5	3.5	7.8	15 min.	1	2.7	6.0	2 hrs.
3	36.8	0				6	8.1	22.0	1 hr.
4	27.1	1	5.0	18.4	1 hr.	4	2.8	10.3	5 hrs.
5	66.3	2	3.3	4.9	30 min.	4	6.5	9.8	2 hrs.
		13				16			

minutes. We have in our work similar fluctuations, the catalytic power of the blood undoubtedly varied in the different samples to the degree recorded, and the real difficulty lies in fitting such fluctuations into a rational explanation of the action of saccharin upon the blood or the fixed cells of the body. It is our opinion that if saccharin has any definite, real effect in increasing the catalytic power of the blood it should appear regularly as a rise in the curve, and should present definite and determinable proportions. Our results are shown in tables 9 and 10 and in figure 6.

In these tables we present the data from 18 experiments performed on 9 dogs to whom saccharin dissolved in water was given in amounts varying from 0.5 to 4.0 gram per kilo by means

of a stomach tube. No anaesthetic was employed either in the bleeding or the administration of the drug. In figure 6 we give graphically the results of each of these experiments. In table

TABLE 7
Influence of NaCl solution intravenously on the catalase content of the blood.

DATE	NUMBER	SEX	WEIGHT	SACCHARIN			BEFORE		AFTER							
				Amount	Grams per kilo	NaCl	24 hrs.	5 min.	15 min.	30 min.	45 min.	60 min.	2 hrs.	5 hrs.	24 hrs.	
6-28	3	M	10	0	0	20	33.2	40.3	38.2	35.7	33.6	29.1	34.0	33.1	40.1	
6-28	5	F	15	0	0	25	69.4	64.5	62.9	66.0	61.7	62.4	59.1	63.2	70.3*	
6-28	6	F	11	0	0	24	25.0	20.2	20.6	20.9	21.3	20.4	21.3	21.3	20.8*	
Average							42.53	41.66	40.56	40.86	38.86	37.30	38.13	39.20	43.72	

* Last reading forty-eight hours after test day.

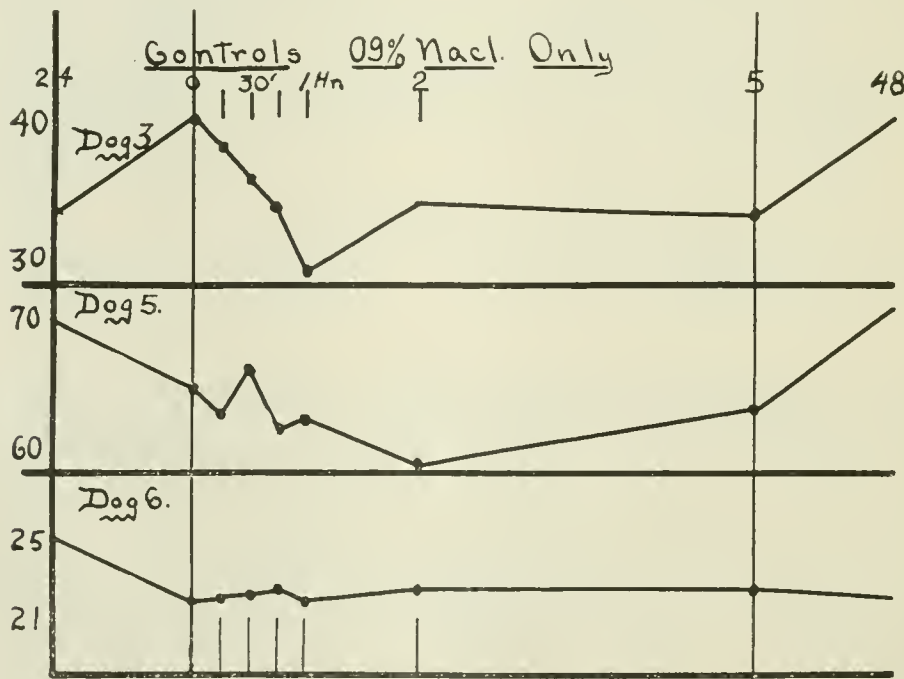


FIG. 5. EFFECT OF SALT SOLUTION ON THE CATALASES OF THE BLOOD

10 we give a summary with special reference to the maximum deviation from the normal both in the positive and the negative direction.

There are many facts to be pointed out: Of the 18 doses mentioned 9 were of 4 grams per kilo. Of these 9 in only 1 case did the animal retain the total amount administered. Thus there was in every case except one some loss of saccharin, and the results are the effect of less than the amount of drug indicated. Thus it is practically impossible in the unanaesthetized animal to determine the full effect of 4 grams of soluble saccharin per kilo. Four of the 9 cases showed also a marked diarrhea. It will be noted that the smaller doses were given in the same way, but did not produce vomiting and diarrhea. Thus the method of administration was not at fault and the symptoms mentioned

TABLE 8
Summary of table 7

EXPERI- MENT	NORMAL	RISES				FALLS			
		Number	Greatest rise			Number	Greatest fall		
			Cubic centi- meters	Per cent	Time		Cubic centi- meters	Per cent	Time
3	40.3	0				6	11.2	27.7	1 hr.
5	64.5	1	1.5	2.3	30 min.	5	5.4	8.3	2 hrs.
6	20.2	6	1.1	5.4	45 min. 2 hrs. 5 hrs.	0			
		7 = 38.8 per cent				11 = 61.1 per cent			

are the result of the action of the drug. There is, therefore, no doubt that saccharin in the amount given by Burge to produce the alleged rise in catalytic power is a gastro-intestinal irritant.

Facts of greater importance from the standpoint of the study of catalases, however, are evident from the summary shown in table 10. In the 108 determinations made on experiment days, rises were observed in 39 instances, 36.1 per cent; falls in 68 instances, 60.19 per cent; and no change in 4 instances, 3.7 per cent. Considering the cases in which the catalase content rose; the greatest rise from the standpoint of percentage was in experiment 1, where the rise was 51.8 per cent, but this great percentage

TABLE 9
Effect of saccharin by mouth on the catalase content of the blood

DATE	NUMBER	SEX	WEIGHT	SACCHARIN			BEFORE		AFTER							REMARKS
				Amount	Grams per kilo	Water	24 hrs.	5 min.	15 min.	30 min.	45 min.	1 hr.	2 hrs.	3 hrs.	24 hrs.	
6-5	1	F	13.0	52	4.0	250	12.6	9.2	9.2	11.3	11.1	12.1	13.9	13.5	11.9	Marked diarrhea after 2 hours
6-5	2a	M	10.0	40	4.0	250	62.8	53.4	56.2	53.4	59.5	58.2	58.2	58.4	56.5	Marked diarrhea after 2 hours
6-16	2b	M	10.0	40	4.0	300	55.0*	42.5	45.2	47.0	45.7	45.5	48.1	39.6	59.5	Vomited one-half after 30 minutes
6-5	3a	M	10.0	40	4.0	200	39.0	28.9	36.4	34.1	27.4	25.5	24.2	28.5	34.9	Vomited one-half after 6 minutes
6-16	3b	M	10.0	40	4.0	300	39.7*	39.3	38.2	36.3	35.4	33.8	39.6	41.1	44.2	Vomited one-half after 30 minutes
8-5	3c	M	12.0	15	1.2	100	33.8	34.7	32.1	30.5	31.8	33.1	33.2	33.1	29.5	No symptoms
6-6	4	M	11.0	40	3.6	300	31.8	33.6	35.3	25.7	36.6	36.4	42.8	41.1	29.6	No symptoms
6-6	5a	F	15.0	60	4.0	300	80.9	80.7	79.2	71.9	71.0	72.9	68.6	73.6	71.0	Vomited one-half after 5 minutes
6-16	5b	F	15.0	41	2.6	200	71.5*	65.2	73.8	67.9	67.9	69.6	63.9	66.6	59.1	No symptoms
8-5	5c	F	15.0	17	1.1	100	76.3	69.3	69.9	69.1	67.1	70.4	71.5	69.7	69.5	No symptoms
8-7	5d	F	15.0	60	4.0	300	69.5	65.5	65.5	59.5	66.2	69.0	72.1	65.2	61.4	No symptoms
8-9	5e	F	15.0	30	2.0	200	61.4	64.8	62.0	62.9	59.8	60.7	52.4	60.7*	64.6	No symptoms
8-7	6	F	11.0	30	2.5	200	54.1	64.2	61.6	58.6	54.1	54.9	52.1	57.3	46.6	No symptoms
8-7	7	F	7.6	4	0.5*	100	55.2	53.2	53.2	50.6	50.6	46.6	45.3	45.3	44.0	No symptoms
8-4	8a	M	11.7	6	0.5*	100	75.8	65.6	62.9	61.3	47.0	55.9	54.6	52.1	56.2	No symptoms
8-7	8b	M	11.7	48	4.0	300	47.1	54.7	53.6	48.0	48.0	49.1	49.9	55.4	51.0	Vomited one-half after 10 minutes.
8-4	9a	F	8.0	4	0.5	100	48.1	36.7	35.6	36.4	36.6	34.7	34.2	31.7	40.6	Diarrhea
8-9	9b	F	8.0	32	4.0	300	35.4	26.5	27.8	23.0	13.7	25.2	23.1	27.6	28.7	No symptoms
Vomited one-half after 30 minutes.																
Diarrhea																
Average.....																

* Average of 4 readings.

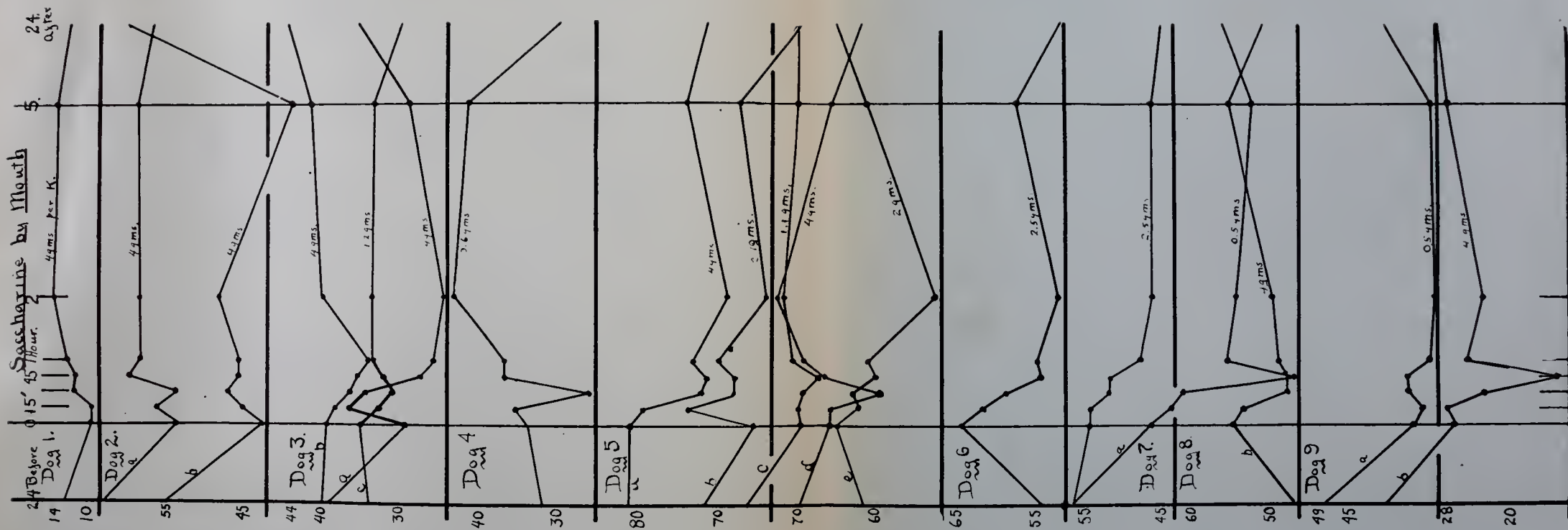


FIG. 6. EFFECT OF SACHARIN BY MOUTH ON THE CATALASES OF THE BLOOD

rise involved only an increase of 4.7 cc. of gas. The greatest rise from the standpoint of volume was in experiment 4 where the increase was 9.2 cc., the percentage increase being 27.2 per cent. From the standpoint of time at which the maximum increase came: the maximum came in 3 instances in fifteen

TABLE 10
Summary of table 9

NUMBER OF EXPERI- MENT	NOR- MAL	RISES				FALLS				NO CHANGE	
		Num- ber	Greatest rise			Num- ber	Greatest fall			Num- ber	Time
			Cubic centi- meters	Per cent	Time		Cubic centi- meters	Per cent	Time		
1	9.2	5	4.7	51.8	2 hrs.	0				1	15 min.
2a	53.4	5	6.1	11.5	45 min.	0				1	30 min.
2b	42.5	5	5.6	13.1	2 hrs.	1	2.9	6.8	5 hrs.	0	
3a	28.9	2	7.5	25.9	15 min.	4	4.7	16.0	2 hrs.	0	
3b	39.3	2	1.8	4.5	5 hrs.	4	5.5	13.9	1 hr.	0	
3c	34.7	0				6	4.2	11.4	30 min.	0	
4	33.6	5	9.2	27.4	2 hrs.	1	7.9	23.5	30 min.	0	
5a	80.7	0				6	12.1	14.9	2 hrs.	0	
5b	65.2	5	8.6	13.1	15 min.	1	1.5	2.3	2 hrs.	0	
5c	69.3	4	2.2	3.1	2 hrs.	2	0.2	0.2	30 min.	0	
5d	65.5	3	6.6	10.0	2 hrs.	2	6.0	9.1	30 min.	1	15 min.
5e	64.8	0				6	5.0	7.7	45 min.	0	
6	64.2	0				6	10.1	15.0	2 hrs.	0	
7	53.2	0				5	7.9	14.8	2 hrs.	1	15 min.
									5 hrs.		
8a	65.6	0				6	13.2	20.1	5 hrs.	0	
8b	54.7	1	0.7	1.0	5 hrs.	5	6.7	1.2	30 min.	0	
									45 min.		
9a	36.7	0				6	2.5	6.8	2 hrs.	0	
9b	26.5	2	1.3	4.9	15 min.	4	12.8	48.6	45 min.	0	
		39 = 36.1 per cent				65 = 60.19 per cent				4=3.7 percent	

minutes, in 1 instance in 45 minutes, in 5 instances in two hours, in 2 instances in five hours. It is, therefore, evident that while increases occur 39 times in 108 tests these increases are usually small from the standpoint of volume, not materially larger than rises observed in the normal controls, nor do they occur more frequently than in the controls, in 44.8 per cent of cases normally,

in 36.11 per cent of cases in the experiment animals just mentioned for all bleedings, or in 44.4 per cent of cases using the five hour observation only. In addition there is no regularity regarding the time when the rise comes. It is thus clear that no prediction can be made regarding time or the duration of the rise.

Considering the cases in which the catalase content fell: the greatest fall from the standpoint of percentage was in experiment 9 b, 48.6 per cent; the greatest fall from the standpoint of volume was in the case of 8 a, 13.2 cc. The maximum decrease came in 4 instances in thirty minutes, in 3 instances in forty-five minutes, in one instance in one hour, in 6 instances in two hours, and in 3 instances in five hours. In 1 case—experiment 7—the two hour and the five-hour readings were the same, and in another—8 b—the thirty-minute and forty-five-minute readings were the same. Thus it is clear that the percentage of falls in catalase power after saccharin is not materially different either in number or amount from similar changes in normal animals without drugs. In observation on dogs bled 7 times without saccharin the catalytic power was decreased in 55.1 per cent of cases; in observations on dogs bled 7 times with saccharin the catalytic power of the blood was decreased in 60.19 per cent of cases. Ruling out all of the observations but the five-hour ones, the catalytic power was decreased in 55.6 per cent of cases whereas in the dogs bled only twice at five-hour intervals falls appeared in 60 per cent of cases.

Hence the conclusion is warranted: *While fluctuations occur in the catalytic power of the blood of animals after the administration of saccharin in amounts varying between 0.5 and 4.0 gram per kilo of body weight those fluctuations are practically identical with the fluctuations in the normal animal. These fluctuations occur as often in the normal as in the test animal, and the changes are just as large. Hence there is no evidence that saccharin produces any change in the catalytic power of the blood of dogs in either the positive or the negative direction.*

We have already pointed out that in 8 of 9 cases where 4 grams of saccharin per kilo were given by mouth the animal showed vomiting, and in 4 cases marked diarrhea. Consequently there

was a loss of saccharin, and the effects recorded were the effects of less than that amount. In order to secure data from experiments where the amount given was actually retained and actually entered the blood stream we injected saccharin in doses varying from 0.6 to 2.5 grams per kilo dissolved in warm 0.9 per cent NaCl solution intravenously. Two of 13 cases were immediately fatal (in two to three minutes) (in another there was profound shock and in two cases vomiting). Dogs 7 and 11 died, dog 3 suffered severe shock during intravenous injection of saccharin. The data from the 11 non-fatal cases are shown in table 11, and the graph in figure 7. In every case about ten seconds after the injection of saccharin was begun the animal gave every evidence of tasting some peculiar substance, smacking and licking the lips was the common manifestation.

As can be seen from the table and the graph in 5 of 11 experiments there was a reduction of the catalytic power in every reading after the normal—in no case was there observed a reading as high as the normal. In 2 of these 11 experiments there was an increase above the normal in every reading of that day after the drug was given. These animals had received a large dose—2 grams per kilo—and 70 cc. of warm NaCl and showed a marked diuresis. Dog 12 voided 400 cc.; dog 13, 500 cc. of urine during the five hours of the experiment, an amount more than the averages of these dogs for twenty-four-hour periods. Taking all the determinations, 54 in number, into consideration rises were observed in 12 cases, 21.0 per cent and falls in 45 cases, 78.9 per cent.

If we compare the results of these experiments on the intravenous injection of saccharin with the controls—dogs bled repeatedly after the injection of 0.9 per cent NaCl solution—we find that a fall in catalytic power of the blood is much more frequent in the former than in the latter—in 78.9 per cent in the experiment as compared with 61.1 per cent in the controls. The cause of these falls is obvious. It has already been pointed out by Matthews and McGuigan that saccharin *in vitro* decreases the power of the blood to release oxygen from hydrogen peroxide. Since in these cases of intravenous injection there was the rapid expo-

TABLE II
Influence of saccharin intravenously on the catalase content of the blood

DATE	NUMBER	SEX	WEIGHT	SACCHARIN			BEFORE		AFTER							REMARKS
				Amount	Grams per kilo	NaCl cc.	24 hrs.	5 min.	15 min.	30 min.	45 min.	1 hr.	2 hrs.	3 hrs.	24 hrs.	
6-12	1	F	13.0	10	0.77	20	18.3	15.1	14.6	13.5	14.0	19.5	24.8	24.8		No symptoms
6-20	2	M	10.0	10	1.0	20	55.2	48.2	43.2	37.6	41.0	41.4	46.2	38.1		No symptoms
6-20	3a	M	10.0	10	1.0	20	43.6	42.5	36.6	38.1	34.1	32.2	37.7	41.8		No symptoms
6-25	3b	M	10.0	10	1.0	25	48.1	47.2	40.4	36.1	33.3	32.2	36.5	43.4	29.9	No symptoms
8-9	3c	M	10.0	25	2.5	100*	28.9	34.8	25.4	25.0	25.5	26.3	28.9	25.5	39.4	Animal collapsed with the injection. Shock profound. Dog moribund next morning
6-12	4	M	11.0	10	0.9	25	23.7	26.2	24.2	23.1	23.5	23.3	23.9	29.5		No symptoms
6-20	5	F	15.0	10	0.6	20	75.3	75.2	71.9	70.8	65.1	67.8	66.5	76.3		No symptoms
6-25	6	F	11.0	11	1.0	20	33.3	29.7	30.7	28.0	27.5	29.0	28.4	27.7	27.0	No symptoms
8-18	8	M	11.7	10	0.85	25		44.6				40.9	44.0	38.4		No symptoms
9-19	12	F	8.0	16	2.0	60	38.2	35.7				37.4	37.6	42.2	31.8	Vomited 5 minutes after injection. Marked diuresis 400 cc. in 5 hours
9-19	13	F	10.0	20	2.0	60	89.0	92.9				94.5	107.3	112.3	104.9	Vomited 1 minute after injection. Marked diuresis 500 cc. in 5 hours
Average of 8 experts.....																
Average of all experts after 1, 2 and 5 hours.....																

* Dog's blood—not salt solution.

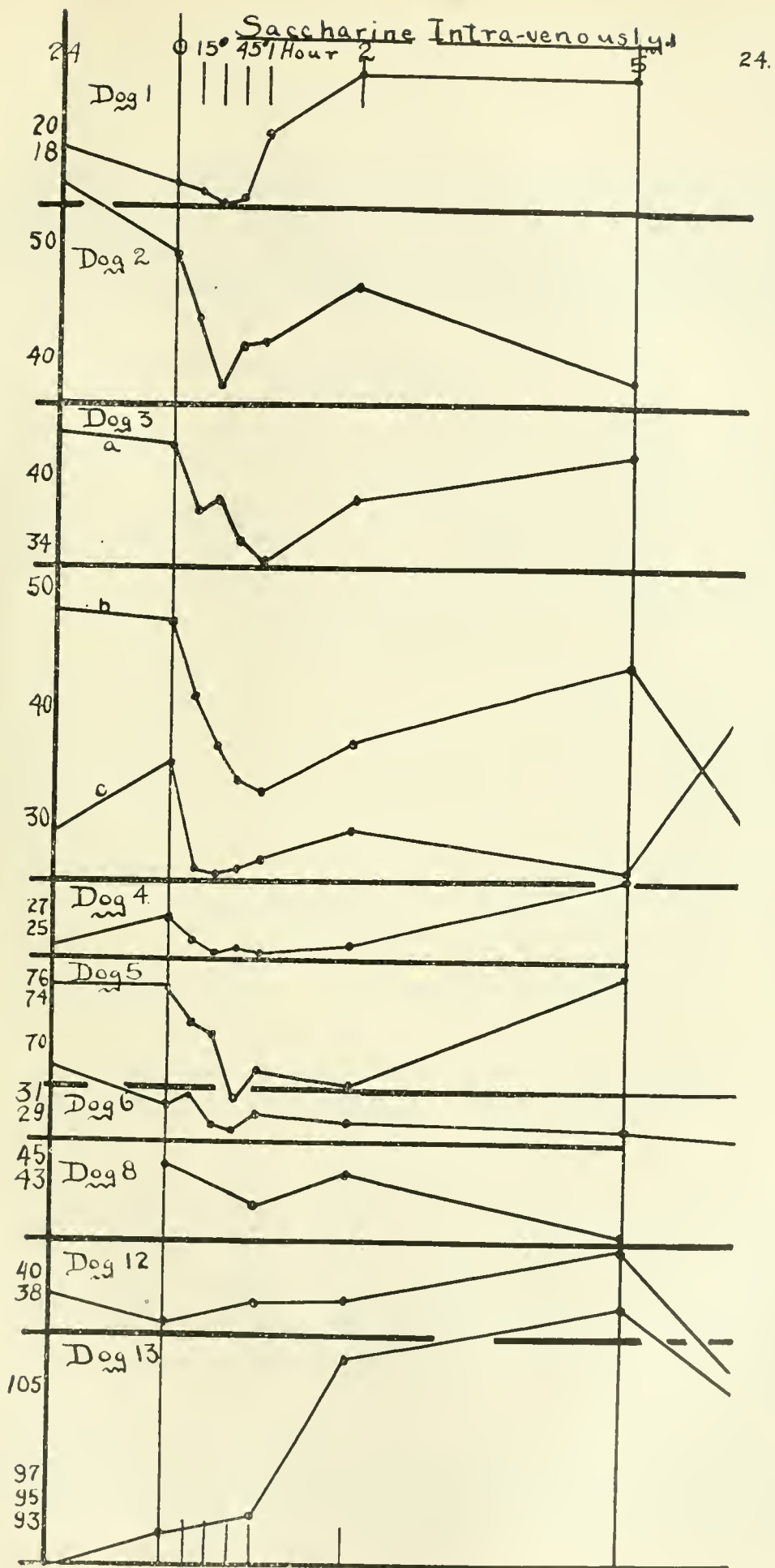


FIG. 7. EFFECT OF SACCHARIN INTRAVENOUSLY UPON THE CATALASES OF THE BLOOD

sure of the blood to relatively large amounts of saccharin, it was perfectly clear that the effect of that drug upon the blood in the test tube must be re-investigated. In table 13 we show the effect of the exposure of the blood to saccharin.

TABLE 12
Summary of table 11

NUMBER OF EXPERI- MENT	NORMAL	RISES				FALLS			
		Number	Greatest rise			Number	Greatest fall		
			Cubic centi- meters	Per cent	Time		Cubic centi- meters	Per cent	Time
1	15.1	3	9.7	64.2	{ 2 hrs. 5 hrs. }	3	1.6	10.6	30 min.
2	48.2	0				6	10.6	21.9	30 min.
3a	42.5	0				6	10.3	24.2	1 hr.
3b	47.2	0				6	15.0	31.8	1 hr.
3c	34.8	0				6	9.8	28.1	30 min.
4	26.2	1	3.3	12.5	5 hrs.	5	3.1	11.8	30 min.
5	75.2	1	1.1	1.4	5 hrs.	5	10.1	13.4	45 min.
6	29.7	1	1.0	3.3	15 min.	5	2.2	7.4	45 min.
8	44.6	0				3	6.2	13.9	5 hrs.
12	35.7	3	6.5	18.2	5 hrs.	0			
13	92.9	3	19.6	21.0	5 hrs.	0			
		12 = 21.8 per cent				45 = 78.9 per cent			

TABLE 13
Effect of varying amounts of saccharin on blood in vitro upon the catalytic power of the blood

SPECIES	SEX	NORMAL	4 PER CENT	2 PER CENT	1 PER CENT	0.5 PER CENT	0.25 PER CENT
Dog.....	M	54.4	49.8	51.8	50.5	51.8	52.6
Dog.....	F	41.7	40.1	41.3	41.2		
Man B.....	M	190.1	170.0	175.0	184.5	186.0	
Man B.....	M	158.7	132.4	143.3	149.9	154.3	
Man X.....	M	128.2	119.8				
Goat.....	F	92.1	85.2	88.4	90.0		

Saccharin solution was added in an amount sufficient to make the percentages indicated at the top of the column. Four per cent is the amount present in the blood of an animal, if 4 grams of saccharin are given, if all enters the blood stream, and if the blood is equal to one-tenth of the body weight.

This series of tests were carried out to reproduce the possible happenings in the animal body. If one-tenth of the body weight is blood then for every kilo of body weight there would be 100 cc. of blood. If 4 grams were to be given per kilo there would be 4 grams for every 100 cc. or a 4 per cent solution. Thus we added to the blood varying amounts of saccharin, enough to make the dilutions shown in the table.

As can be seen from the table there is decreased catalytic power of the blood following the action of saccharin, and the decrease is greatest where the most saccharin is present. Thus the decrease is proportional to the amount of saccharin present in the blood. It is also to be noted that decreases are more marked in bloods with higher catalytic power. From this data it is perfectly clear that if saccharin were to enter the blood stream in large quantities—2 to 4 grams per 100 cc.—the direct effect of the drug would be to decrease rather than to increase the catalytic power. In our experiments we saw no indication that in a low concentration saccharin increased the catalytic power. That this factor is active is clear from the fact that animals normally between 9.00 a.m. and 2.00 p.m. show a fall in only 55.1 per cent of cases; if given NaCl solution only in 61.1 per cent of cases; if given saccharin and NaCl they show a fall in 78.9 per cent of cases. Thus we are convinced that the fall observed in the catalytic power of the blood of dogs after the intravenous injection of saccharin is due at least in part to the direct action of the drug upon the catalytic power *in vivo* as well as *in vitro*.

One further point must be mentioned: Dogs 12 and 13 were the only two animals in which a rise was observed consistently after the intravenous injection of saccharin, and both these animals showed a marked diuresis. The rise in these cases may have been due not so much to the increase of the actual total amount of catalase as to the concentration of the blood from the diuretic effect of the saccharin or of the salt solution in which it was dissolved.

The conclusion we draw from this part of the work is that *saccharin intravenously reduces the catalytic power of the blood, unless a diuresis follows its administration.*

In order to determine whether the catalase content varied with the red count after saccharin two experiments were undertaken on an animal in which these two factors were studied simultaneously. The results of these two experiments are shown in table 14 and figure 8.

TABLE 14

Effect of the administration of saccharin upon the catalytic power and the erythrocyte count of dogs

DATE	NUMBER	SEX	WEIGHT	SACCHARIN				BEFORE		AFTER					
				Total	Grams per kilo	Water or salt	Method	O ₂	Reds	1 hour		2 hours		5 hours	
										O ₂	Reds	O ₂	Reds	O ₂	Reds
9-18	12	F	8K	16	2	60	Intra-ve-nous	35.7	7,360,000	37.4	8,000,000	37.6	8,000,000	42.2	9,600,000
9-23	12	F	8	32	4	125	Stom-ach	30.5	5,680,000	30.7	5,760,000	30.7	5,600,000	30.3	5,840,000

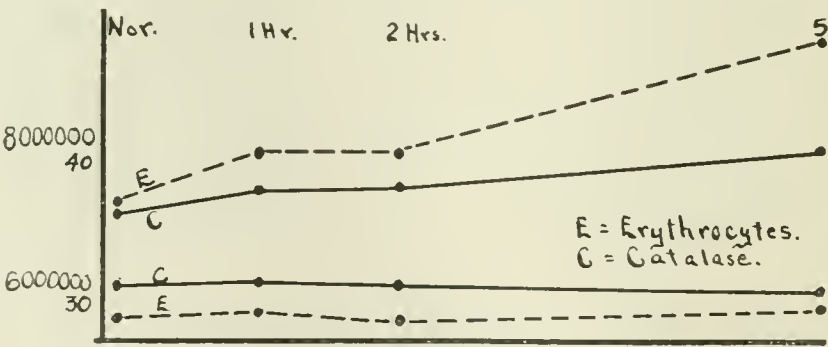


FIG. 8. RELATION BETWEEN THE ERYTHROCYTE COUNT AND THE CATALASE CONTENT OF THE BLOOD

It is perfectly clear from table 14 and figure 8 that the catalase content and the erythrocyte count varied in the same direction in practically every instance. Where the drug was given by mouth there was little change in the red count, or in the catalytic activity. Where the drug was given intravenously there was either marked increase both in the red count and in the catalytic activity and these changes ran parallel with each other. It is to be noted in the last case that the dog showed a marked

diuresis, voiding more urine during the four hours of the experiment than during preliminary twenty-four hour periods. It is quite clear that this diuresis will explain both the increased count and the increased catalytic activity. There is not enough data in the experiments to be more than suggestive, but there is no doubt in the mind of the writer that similar changes would be observed if a large number of experiments were to be carried out, for we believe that the catalase variations are determined by the number of red cells in the circulating blood.

If this is the case and the evidence offered by other writers (6) (7) points to the same conclusion it is rather difficult to understand the mechanism by which the liver increases the catalase content. So far as the evidence goes there is nothing to point to the liver as a source of red cells in extra-uterine life. Hence if the liver is concerned it must be assumed that the ferment is picked up by the cells of the blood as they pass that organ for it is well known that the catalase is confined to the cells of the blood, the plasma being entirely inactive. Further, Stehle has failed to confirm the findings of Burge regarding the differences between jugular and hepatic blood, finding that they run parallel instead of the catalase content being lower in the latter than in the former; therefore, the mechanism by which the liver increases the catalase content of the blood need cause no further worry.

THE CATALASES IN DIABETES

Dogs

Having shown that saccharin in the dog has no effect upon the catalytic power of the blood, for the variations are in the same direction and are equal to the changes in the controls, the effect of the removal of the pancreas was studied. Removal of the pancreas has no constant effect upon the catalytic power of the blood of animals as a comparison of the catalytic power of the blood in table 16 with the normal in the same animal in the preceding tables shows. In some cases removal of the pancreas with the subsequent diabetes increases, in some case decreases

TABLE 15
Effect of saccharin on the catalytic power of the blood in pancreatic diabetes in the dog

DATE	NUMBER	SEX	WEIGHT	SACCHARIN				BEFORE		AFTER								REMARKS
				Amount	Grams per kilo	NaCl or water		24 hrs.	5 min.	15 min.	30 min.	45 min.	60 min.	2 hrs.	3 hrs.	4 hrs.	24 hrs.	
8-8	5	F	12.5	50	4.0	200			91.6	92.2	94.2	94.1	92.9	84.7				Under ether oesophagus tied. Drug injected into stomach and bowels
8-19	6a	F	10.0	10	1.0	10			45.1				38.3	46.4		45.1	41.2	Intravenously
9-3	6b	F	10.0	40	4.0	200			36.7				38.4	35.6		37.1		Animal vomited one-half in 4 minutes
9-10	6c	F	10.0	10	1.0	15			46.5				28.2	43.2		46.1	26.0	Intravenously. Animal vomited in 4 minutes
9-15	6d	F	10.0	20	2.0	70		46.7	45.9				43.8	47.7		46.6	41.6	Intravenously. Animal vomited in 3 minutes
9-3	6e	F	10.0	45	4.5	125		40.8	42.1				38.4	38.9		42.9		Animal under ether. Oesophagus tied
8-25	8	M	10.0	40	4.0	125		52.4	55.9				60.3	55.9	46.4			Oesophagus tied. One-half in bowel. Animal under ether one-half in stomach
8-30	9	F	8.0	26	3.0	100		41.9	50.2				54.5	42.4				Sugar 5 per cent. Urine 200 cc.
9-25	13	F	10.0	20	2.0	70			99.7	94.2	69.1	82.4	86.7	106.4		110.6		Intravenously. Dog vomited repeatedly
Average									57.08				53.50	55.69				

the catalytic power. It must be remembered that these animals are decidedly ill from the operation, and are unable to retain water, hence although an increased catalytic power is shown in some cases, increased concentration of the blood because of increased output with reduced intake of fluid is a perfectly adequate and satisfactory explanation. We are of the opinion that the variations are within the normal limits of variation for animals subjected to operation.

TABLE 16
Summary of table 15

EXPERI- MENT	NORMAL	RISES				FALLS			
		Number	Greatest rise			Number	Greatest fall		
			Cubic centi- meters	Per cent	Time		Cubic centi- meters	Per cent	Time
	cc.								
5	91.6	4	2.6	2.8	30 min.	1	6.9	7.5	2 hrs.
6a*	45.1	1	1.3	2.8	2 hrs.	1	6.8	12.8	60 min.
6b	36.7	2	1.7	4.6	60 min.	1	1.0	2.7	2 hrs.
6c	46.5	0				3	18.3	39.3	60 min.
6d	45.9	2	1.6	3.4	2 hrs.	1	2.1	4.5	60 min.
6e	42.1	1	0.8	1.9	60 min.	2	3.7	9.0	60 min.
8*	55.9	1	4.4	7.8	60 min.	1	9.5	16.9	3 hrs.
9	50.2	1	4.3	8.5	60 min.	1	7.8	15.5	2 hrs.
13	99.7	2	10.9	10.9	4 hrs.	4	30.6	30.6	30 min.
		14 = 45.7 per cent				15 = 48.4 per cent			

* No change in 1 case. Total 2 or 6.4 per cent.

In tables 15 and 16 figure 9 we show the results obtained from 9 experiments on 5 animals with pancreatic diabetes. The data on this part of the work is not satisfactory because of the state of the animals. If the drug is given by mouth it is vomited immediately; further the animals are in such condition that in most cases the withdrawal of blood at frequent intervals as was the case in the normal dogs is impossible. Further the animals withstood ether poorly, and hence the observations conducted under this anaesthetic were of short duration.

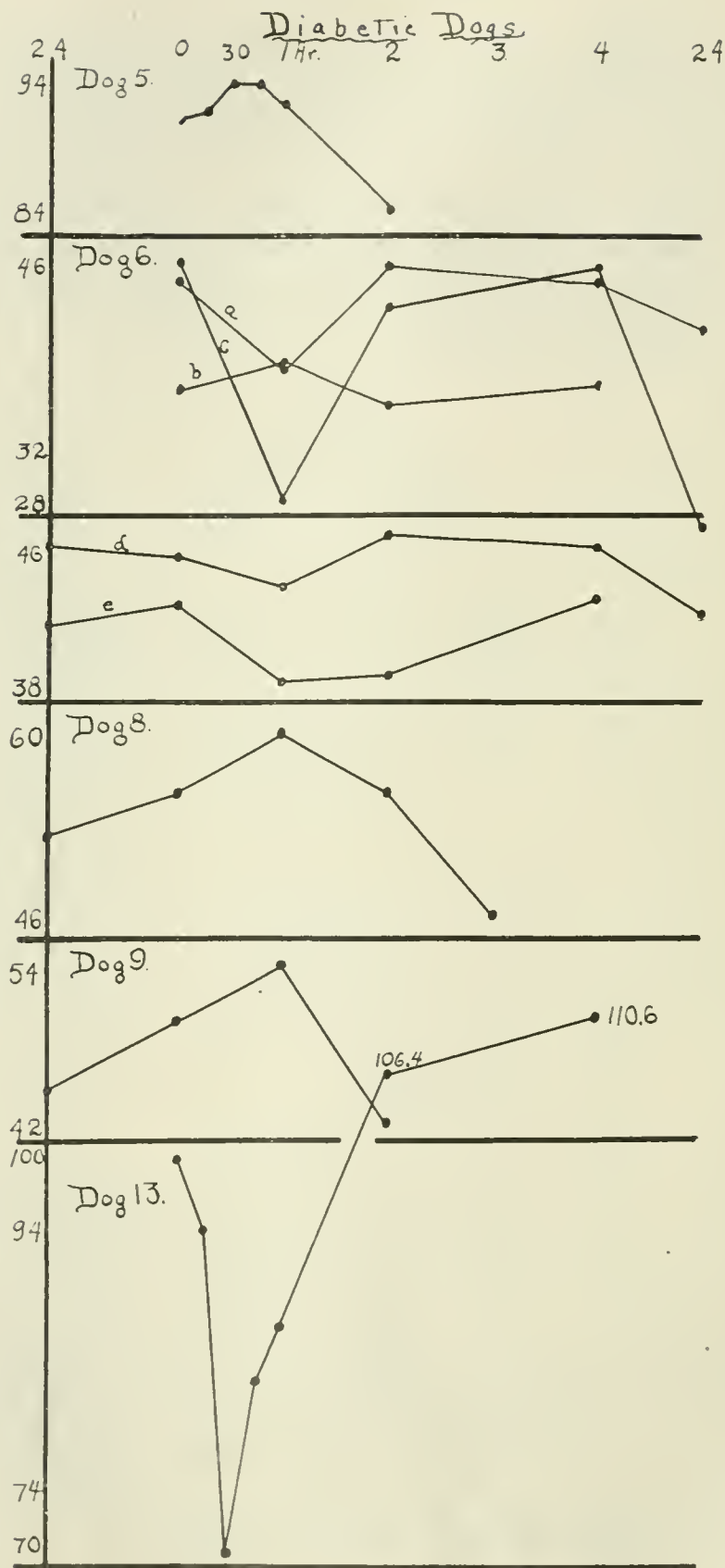


FIG. 9. EFFECT OF SACCHARIN ON THE CATALASE CONTENT OF THE BLOOD OF DIABETIC DOGS

As can be seen from table 17 the catalytic power rose following saccharin in 14 of 31 cases or in 45.7 per cent, it fell in 15 of 31 cases or in 48.4 per cent, and remained unchanged in 2 or in 6.4 per cent. The greatest rise was 10.9 cc. or 10.9 per cent in experiment 13; the greatest fall was 30.6 cc. or 30.6 per cent in the same animal. It would seem remarkable that if these changes were due to the pure action of the drug upon catalases that the greatest rise and the greatest fall should be seen in the same animal. While an increase in the catalytic power of the blood is seen in a slightly larger percentage of cases (45.7 per cent) in the animal with diabetes than in the normal (about 40 per cent),

TABLE 17
Summary of tables 3 to 16

EXPERIMENT	RISES				FALLS			
	Percent of rises	No. of rises	Greatest rise		Percent of falls	No. of falls	Greatest fall	
			Cubic centi-meters	Percent			Cubic centi-meters	Percent
1. Normal 2 bleedings.....	40.0	12	27.2	48.6	60.0	18	21.5	33.0
2. Normal 7 bleedings.....	44.8	13	7.4	44.3	55.1	16	8.1	22.0
3. Normal NaCl only.....	38.8	7	1.5	5.4	61.1	11	11.1	27.2
4. Saccharin by mouth all observations.....	36.11	39	9.2	51.8	60.19	65	13.2	48.6
5. Saccharin intrave- nously.....	16.6	9	19.6	64.2	83.3	45	15.0	31.8
6. Diabetic dogs.....	45.7	14	10.9	10.9	48.4	15	30.6	30.6

we believe that this increase is too slight to be significant and that if as extensive a series of experiments were to be carried out on diabetic as was carried out on normal animals the figures would check very closely. As it is the variations are only slightly different from the normal, and we conclude that saccharin administered intravenously or by mouth to an animal suffering from pancreatic diabetes does not increase the catalytic power of the blood more frequently than the increase is observed in the normal animal. The changes recorded by Burge are the exception and not the rule, since falls follow the administration of saccharin more frequently than rises.

TABLE 18
Effect of saccharin and bleeding on diabetes mellitus

NUMBER	SEX	AGE	CLINICAL DIAGNOSIS	CONDITION OF URINE ON DAY OF EXPERIMENT	SACCHARIN			
					Before	After		
						1 hour	2 hours	3 hours
(1) 709760	F	46	Diabetes melli- tus, cystitis	++1.3%	144.0	142.6	148.0	158.5
(2) 709056	F	60?	Diabetes melli- tus, arthritis	0	83.4	85.1	80.6	74.7
(3) 709191	F	43	Diabetes melli- tus; had had foot ampu- tated — gan- grene	0	143.6	144.5	148.9	138.4
(4) 709598	F	69	Diabetes melli- tus	++	125.3	131.5	131.5	138.6
(5) 709466	M	45	Diabetes melli- tus	++*	116.2	127.0	116.5	121.4
(6) 710122	M	54	Diabetes melli- tus	++2%	119.6	115.4	110.9	114.7
(7) 710937	F	66	Diabetes melli- tus, pruritis vulvae	+++	116.5	121.4	117.3	119.4
Average					121.22	123.92	121.96	123.67

* This patient has been sugar free for 11 days preceding the experiment.

TABLE 19
Summary of table 18

EXPERIMENT	NORMAL CUBIC CENTI- METERS	RISES				FALLS			
		Number	Greatest rise			Number	Greatest fall		
			Cubic centi- meters	Per cent	Time		Cubic centi- meters	Per cent	Time
1	144.0	2	14.5	10.0	4 hrs.	1	1.4	9.0	1 hr.
2	83.4	1	1.7	2.0	1 hr.	2	8.7	10.4	4 hrs.
3	143.6	2	5.3	3.6	2 hrs.	1	5.2	3.6	4 hrs.
4	125.3	3	13.3	10.6	4 hrs.	0			
5	116.2	3	10.8	9.3	1 hr.	0			
6	119.6	0				3	8.7	7.2	2 hrs.
7	116.5	3	4.9	3.3	1 hr.	0			
		14 = 66 per cent				7 = 33 per cent			

The identity of diabetes in man and pancreatic diabetes in animals has not yet been shown, therefore it was considered important that the former condition also be investigated so far

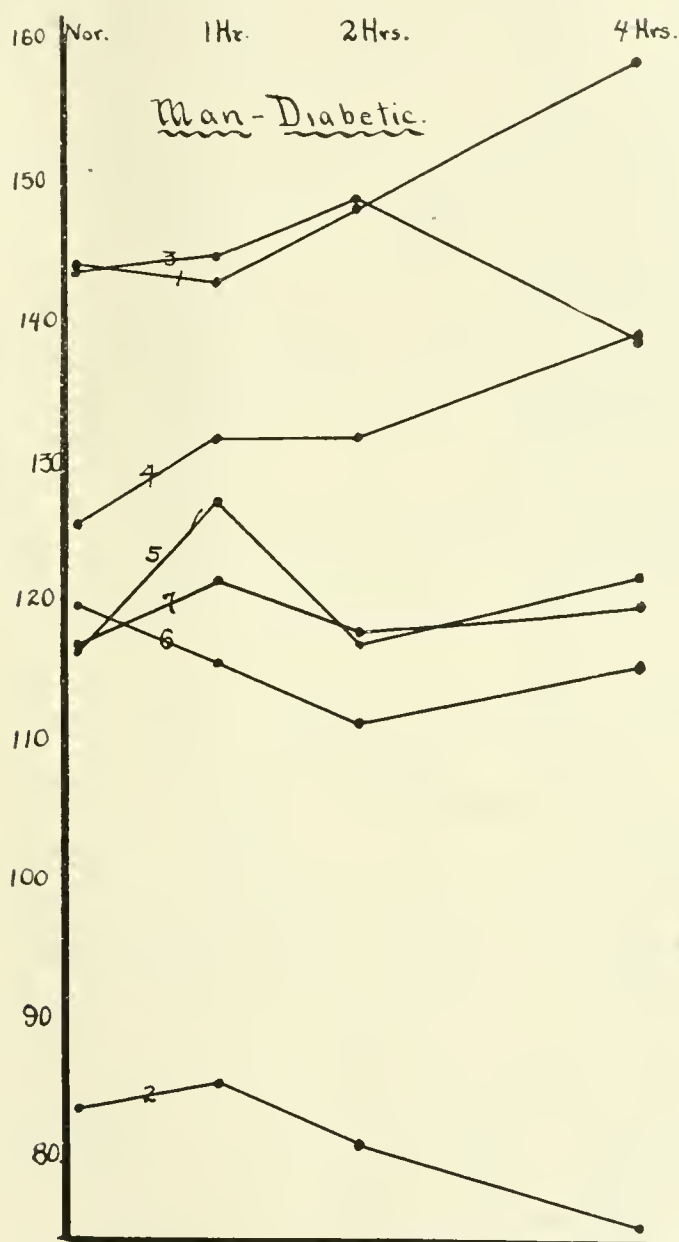


FIG. 10. EFFECT OF SACCHARIN ON THE CATALASE CONTENT OF THE BLOOD OF MAN (DIABETIC)

as the influence of saccharin upon the catalytic power of the blood is concerned. The patients studied were diabetics and non-diabetics in a large charity hospital. The dose used was

1 gram—three times the pharmacopoeial dose—of saccharin given in capsules immediately after the first sample of blood was drawn. This dose for the average man per kilo of body weight was only about $\frac{1}{240}$ of the dose given by Burge to dogs to increase the catalytic power. The cases were all clearly diabetes mellitus, five of the seven had sugar in the urine on the day of the experiment, the other two had been rendered sugar free only a few days previously by careful dieting. There were absolutely no subjective symptoms in any case, either in the diabetics or in the non-diabetic controls.

In tables 18 and 19, and in figure 10 can be seen the results of the experiment on diabetic, in tables 20 and 21, and figure 11 on non-diabetic patients. After saccharin by mouth the catalase content was higher in 66 per cent and was lower in 33 per cent of diabetic cases, using the catalase content of the blood before the administration of the drug as the normal. Under the same conditions the catalase content fell in 79 per cent, and rose in 20.8 per cent of cases in the non-diabetic. These changes were larger as regards volume but no larger as regards percentage than in similar conditions in animals. While this effect in diabetic cases is markedly different from our findings on normal animals and also from non-diabetic cases in man, it is not believed that the observation is of any significance whatever, for it is believed that a longer series under better control would show that the variations were entirely within normal limits. Furthermore even if it were to be established as a fact that the catalase content of the blood were increased after the administration of saccharin the necessary proof that the catalases are concerned in the processes of oxidation within the body is still lacking. Further the amount administered was much smaller— $\frac{1}{240}$ as much—than that needed by Burge to increase the catalytic power of the blood of dogs. Further the averages of the three observations made varied less than 3 cc. (about 2 per cent) from the normal. (See averages of table 18.)

TABLE 20

Effect of saccharin and bleeding on non-diabetic cases

NUMBER	SEX	AGE	CLINICAL DIAGNOSIS	SACCHARIN			
				Before	After		
					1 hour	2 hours	4 hours
(1) 713233	M	61	Scurvy*	105.0	107.7	106.1	105.4
(2) 713243	M	50	Chronic interstitial nephritis	105.6	99.0	95.1	88.2
(3) 713821	M	30	Lead colic	98.5	93.2	88.6	78.7
(4) 713973	M	35	Neurasthenia*	171.8	171.6	170.1	166.2
(5) 714188	M	37	Incipient pulmonary* tuberculosis	142.2	137.0	116.6	125.0
(6) 714303	M	57	Alcohol (wood)* poisoning	141.1	139.1	139.8	143.0
(7) 714397	M	27	Tonsillitis*	136.9	124.8	126.7	122.5
(8) 714459	M	21	Dementia* praecox	111.0	109.9	119.8	106.2
Average				126.51	122.78	120.35	116.9

* These men were practically well physically.

TABLE 21

Summary of table 20

EXPERIMENT	NORMAL	RISES				FALLS			
		Number	Greatest rise			Number	Greatest fall		
			Cubic centimeters	Per cent	Time		Cubic centimeters	Per cent	Time
1	105.0	3	2.7	2.5	1 hr.	0			
2	105.6	0				3	17.4	16.4	4 hrs.
3	98.5	0				3	19.8	20.2	4 hrs.
4	171.8	0				3	5.6	3.2	4 hrs.
5	142.2	0				3	25.6	18.0	2 hrs.
6	141.1	1	1.9	1.0	4 hrs.	2	2.0	1.0	1 hr.
7	136.9	0				3	14.4	10.5	4 hrs.
8	111.0	1	8.8	7.9	2 hrs.	2	4.8	4.3	4 hrs.
		5 = 20.8 per cent				19 = 79.1 per cent			

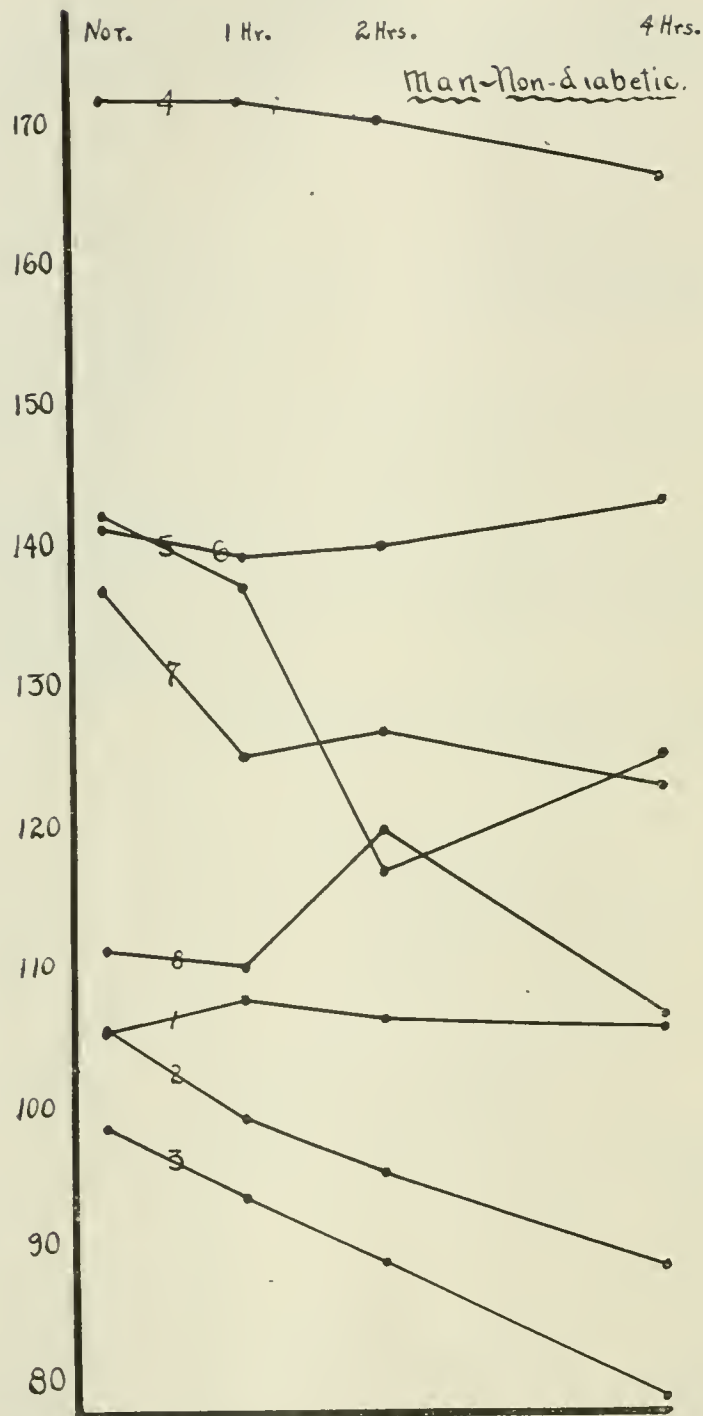


FIG. 11. EFFECT OF SACCHARIN ON THE CATALASE CONTENT OF THE BLOOD OF MAN (NON-DIABETIC)

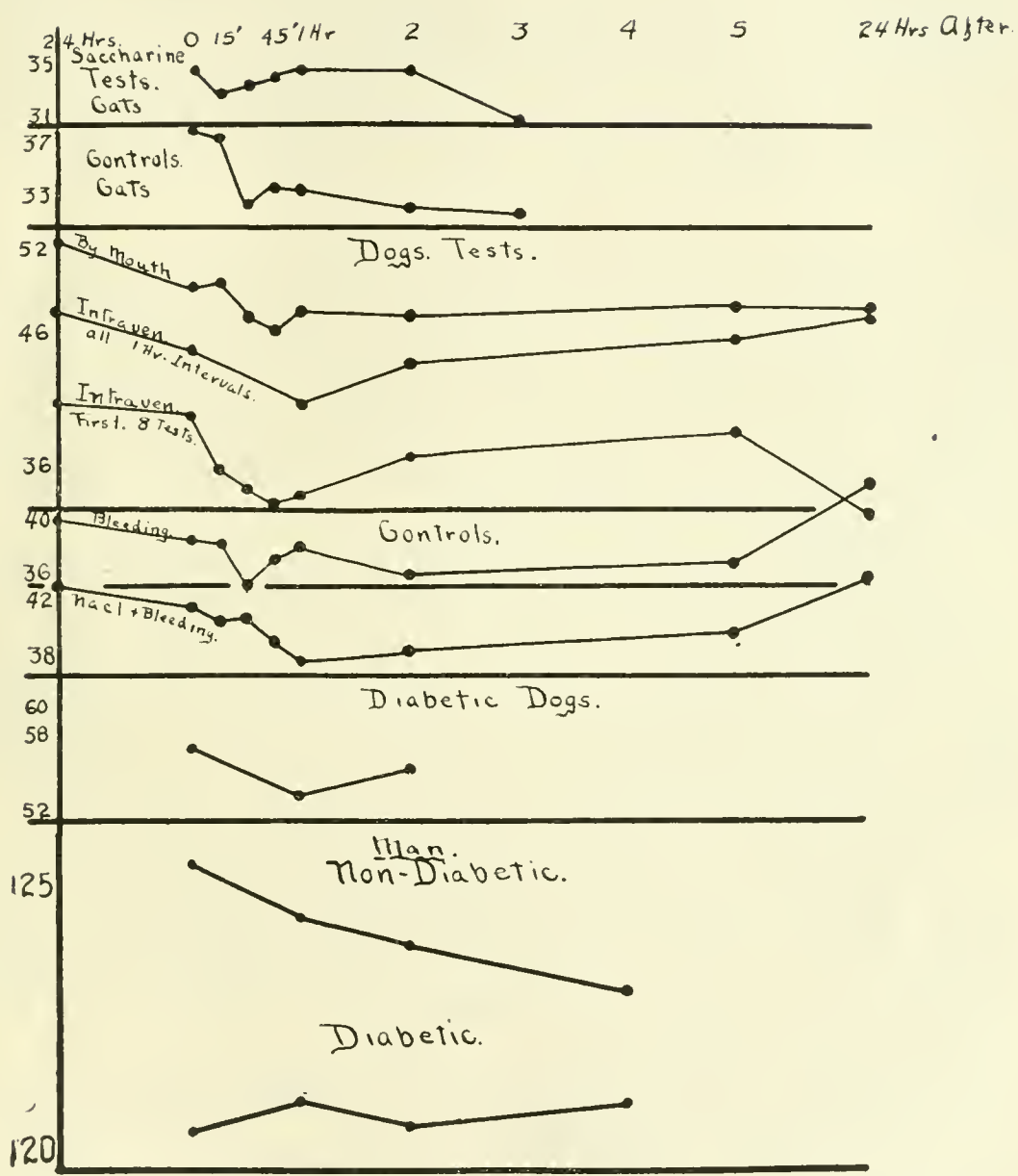


FIG. 12. SUMMARY OF ALL EXPERIMENTS ON THE CATALASE CONTENT OF THE BLOOD

CONCLUSIONS

1. Four grams of saccharin per kilo of body weight does not increase the catalytic power of the blood of cats when the drug is injected into the gastrointestinal canal.

2. Wide variations in the catalytic power of the blood were noted in two dogs studied over a period of 73 to 91 days.

3. Daily variations in the catalase content of the blood are wide.

4. Saccharin in doses of 4 grams per kilo acts as a powerful gastrointestinal irritant producing in most cases vomiting and in many cases diarrhea. The changes in the catalase content were within the limits of normal variation. The drug has no specific action in increasing the catalase content of the blood. Smaller doses show less gastrointestinal irritation but no effect on the catalase content of the blood.

5. Saccharin intravenously produced in practically every case a marked decrease in the catalase content of the blood.

6. Saccharin present in amounts varying between 4 per cent and 0.5 per cent decreases the catalytic power of the blood in vitro. Thus the reduction in the catalytic power of the blood in animals injected intravenously with saccharin is probably due to the direct action of the drug upon the cells of the blood.

7. Since the red corpuscles and the content of catalase varied in the same direction and proportionally in experiments where changes were noted, in all probability the mechanism of action of saccharin is mainly by alteration of the number of red cells per unit volume of the blood.

8. Removal of the pancreas has no specific influence upon the catalase content of the blood.

9. The action of saccharin is the same in the animal with pancreatic diabetes as in the normal animal.

10. An increase was observed in the catalytic power of the blood in 80 per cent of observations of patients with diabetes mellitus. Since the dose was so small and since the averages of all the changes were so small, about 2 per cent, and since there were relatively few cases it is believed that this is of no significance.

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QUANTITATIVE STUDIES IN CHEMOTHERAPY

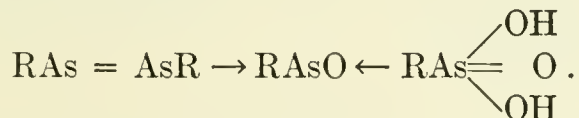
III. THE OXIDATION OF ARSPHENAMINE

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In the first of this series of communications we pointed out that the trivalent oxides of the general type $RAsO$ were the only forms of arsenic which exerted any direct toxic action either upon protozoal parasites or upon the host (1). Other forms, such as arsenobenzol derivatives, $RAs = AsR$, and pentavalent arsenicals, $RAs(O)(OH)_2$, are without direct injurious action except as they are converted by oxidation or reduction respectively, into the trivalent oxide type:



It has long been recognized that the administration of old arsphenamine or neoarsphenamine solutions to patients or animals was attended by considerable danger because of the possibility of increased toxicity due to the oxidation of the arsenobenzol compound into the corresponding oxide. We have therefore thought it advisable to include in this series a study of the oxidation of arsphenamine and the compounds related to it, in hope that such a study might prove of both theoretical and practical interest.

Most organic arsenic preparations are moderately soluble in alkaline solution, due to the formation of the salts of the substituted arsenious or arsenic acids, and, in the case of the phenolic compounds, to the phenolate. Practically all of them are less soluble in a neutral solution, some, including arsphenamine, being

practically insoluble. Only those compounds which contain amino-groups attached to the phenyl radical are appreciably soluble in acid solution.

In the study of oxidations, the hydrogen-ion concentration of the solution in which the oxidation is taking place is of extreme importance, for very few, if any, substances are known which oxidize at a rate independent of the pH. It is a difficult matter to buffer solutions containing arspenamine satisfactorily, since the phenolic group has a very weak acidic nature and the sodium salt undergoes hydrolysis in aqueous solution, with the consequent formation of a large excess of hydroxyl ions. If this excess of hydroxyl ions is reduced by the addition of a strong acid, precipitation of the compound results. The weakly acidic nature of this compound gives it a strong buffering value of its own, and since any buffer used must have a strongly alkaline reaction (pH 9.0–10.0) far removed from the region of biological variations, its use would offer no especial advantages. We have therefore varied the pH by the addition of sodium hydroxide in molecular proportions and believe that by this method we have disclosed such relationships between pH and rate of oxidation as are of any consequence.

METHODS

Solutions of the various compounds were oxidized by a current of air, the course of oxidation being followed by titrating portions of the solutions at appropriate intervals with standard iodine. Since many aromatic compounds absorb iodine in neutral or alkaline solution, it is necessary to acidify the portion to be titrated with HCl. Oxidation of trivalent arsenic is stopped in acid solution, so that this procedure has the added advantage of permitting the samples to be drawn and acidified, and titrated at leisure.

Air was drawn through a strong KOH solution to remove CO₂, then through an oil flow-meter to indicate the volumes-per-minute flow, and then through two ordinary gas-washing bottles placed in series in a water bath which was kept at 38° ± 0.5. The first of these washing bottles contained distilled water to saturate the air with water-vapor at the temperature of the bath, and

the second contained the solution to be oxidized. Negative pressure was maintained by a water vacuum pump. The water bath was about 14 inches deep and made of metal, so that the solution in the oxidizing cylinder was in virtual darkness throughout the experiment. The flow of air was so adjusted that the solution was constantly saturated. Five hundred cubic centimeters per minute were found sufficient to give concordant results with the concentrations studied.

EXPERIMENTAL.

Arsenious acid, methyl arseniousoxide, ethyl arseniousoxide, phenyl arseniousoxide, p-amino phenyl-arseniousoxide and diphenyl arseniousoxide are not oxidized appreciably by atmospheric oxygen when the theoretical amount of NaOH is added to form the monosodium salt. With three or four times this quantity of NaOH in excess, there is very gradual oxidation amounting to 10 to 15 per cent in two to three hours. No differences were noted in the behavior of these compounds, and they may be considered as relatively stable towards atmospheric oxygen.

m-amino-p-oxyphenylarseniousoxide, "arsenoxide." The influence of alkali upon the rate of oxidation of this compound is illustrated in chart 1, in which the data of table 1a-f have been plotted. The hydrochloride and the monosodium salt are both extremely stable, the latter showing no oxidation in two hours. The concentration studied was 1 m/L.¹ Consequently, 2 m/L NaOH were required to convert the hydrochloride into the sodium salt. The addition of another milliequivalent per liter renders the compound susceptible to oxidation, though the process is still extremely slow. A further addition of alkali indicates that a maximum velocity is reached with about 4 m/L NaOH. The relative rate of reaction has been estimated by interpolating the time required for 50 per cent completion of the process (table 1f), and the reciprocals of these values have been plotted in chart 3. The nature of the curves would indicate that the process

¹ Concentrations are expressed in milliequivalents per liter (m/L). 1 m/L would be one thousandth molecular solution.

is catalyzed by some reaction product, since the reaction velocity shows a constant acceleration until about 50 per cent of the oxidation is complete. The nature of this catalyst is suggested by the darkening of the solution at the time when the reaction begins to speed up. *P-amino phenyl arsenious oxide* is not oxidized under similar conditions. This compound differs from "arsenoxide" in the position of the amino group, and the absence of the phenolic group. A solution of "arsenoxide" darkens to a dirty

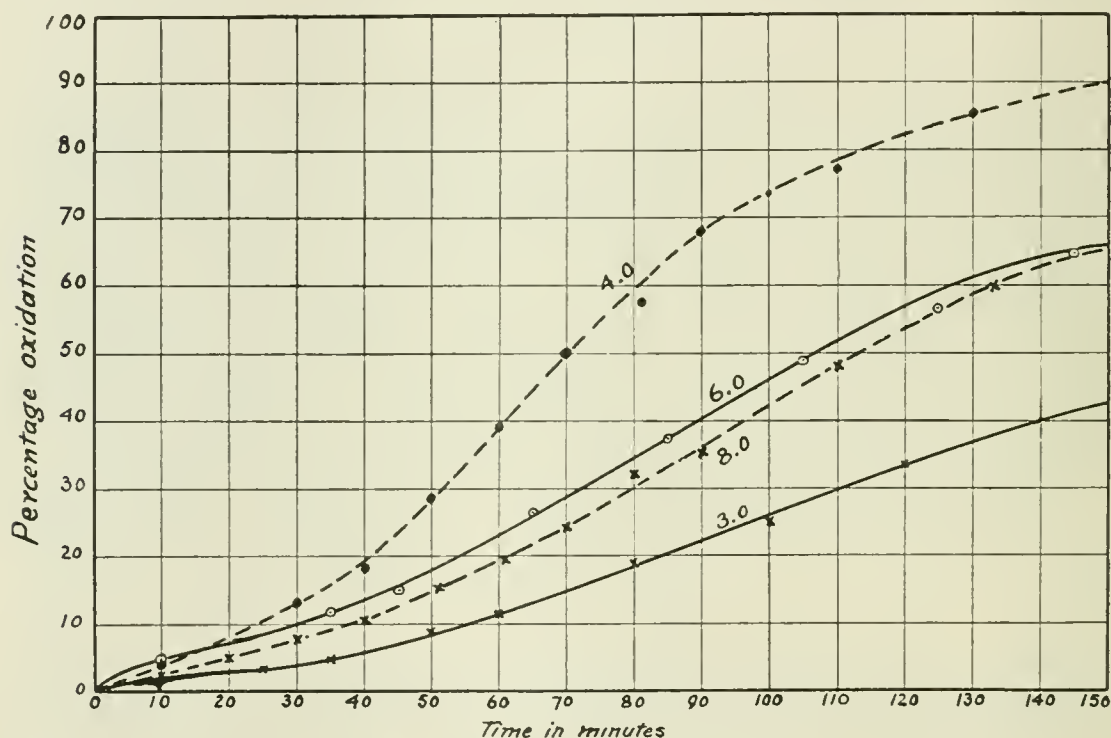


CHART 1. OXIDATION OF M-AMINO-P-OXYPHENYL ARSENIOS OXIDE. 1 m/L

The figures on the curves give the concentration of NaOH in milliequivalents per liter. Note the appearance of acceleration as the oxidation progresses, indicating positive catalysis by some product of the process.

brown as the oxidation proceeds; and in alkaline solution this compound consumes a greater amount of iodine than would be required to oxidize only the arsenic, indicating oxidation in the o-aminophenol group. No color change results from the prolonged exposure of the *p-amino phenyl arsenious oxide*, nor does this compound consume more than the theoretical amount of iodine in alkaline solution so that it is evident that no oxidation

TABLE 1

(a) 1 m/L m-amino-p-oxyphenylarseniousoxide hydrochloride ("arsenoxide"),
2 m/L NaOH. No oxidation in two hours

TIME IN MINUTES	PERCENTAGE OXIDATION
(b) 1 m/L "arsenoxide" hydrochloride, 3 m/L NaOH	
10	2.5
25	3.2
35	4.7
50	9.0
60	11.5
80	19.0
100	25.5
120	33.5
140	39.0
160	43.5
180	47.5
(c) 1 m/L "arsenoxide" hydrochloride, 4 m/L NaOH	
10	3.5
20	5.0
30	13.0
40	18.0
50	28.5
60	39.0
70	50.0
81	57.8
90	68.0
100	73.2
110	76.8
130	85.0
212	92.5
(d) 1 m/L "arsenoxide" hydrochloride, 6 m/L NaOH	
10	4.0
20	4.0
35	11.6
45	14.5
65	26.2
85	37.0
105	49.0
125	56.0
145	64.4

TABLE 1—Continued

TIME IN MINUTES		PERCENTAGE OXIDATION
(e) 1 m/L "arsenoxide" hydrochloride, 8 m/L NaOH		
10		2.5
20		5.0
30		8.0
40		10.5
51		15.0
61		19.7
70		24.0
80		32.0
90		35.0
110		48.2
133		50.0
193		84.0
M/L NaOH	50 PER CENT CONVERSION TIME IN MINUTES	RATE OF REACTION
(f) Rate of oxidation, "arsenoxide"		
2.0*	∞	∞
3.0	200	0.005
4.0	70	0.0143
6.0	110	0.0091
8.0	113	0.00885

* This is the sodium salt.

of the amino group takes place, due either to the absence of the phenol group, or to the para position of the amino group on the ring. That the oxidation of the o-aminophenol group may proceed independently of the oxidation of the arsenic is shown by the rapidity with which *m-amino-p-oxyphenylarsonic acid* darkens and consumes iodine in alkaline solution in a similar manner to "arsenoxide." It would seem that the addition of the former compound to the *p-amino-phenylarseniousoxide* might possibly bring about the oxidation of the latter. No effect was observed, however, when the pentavalent arsenical was present in equimolecular proportions. It seems probable therefore that the oxidation of the arsenic of "arsenoxide" is effected during the simultaneous intermolecular oxidation in the o-aminophenol group.

Arsphenamine. The influence of the hydroxyl ion concentration upon the oxidation of arsphenamine was studied in a similar manner as with "arsenoxide" (table 2a-m, chart 2). The hydrochloride and the monosodium salt are very stable towards atmospheric oxygen. Slow oxidation begins when a slight excess of alkali above the monosodium salt has been added. Unlike "arsenoxide," there is no optimum hydroxyl ion concentration, but the rate of reaction continually increases with increasing addition

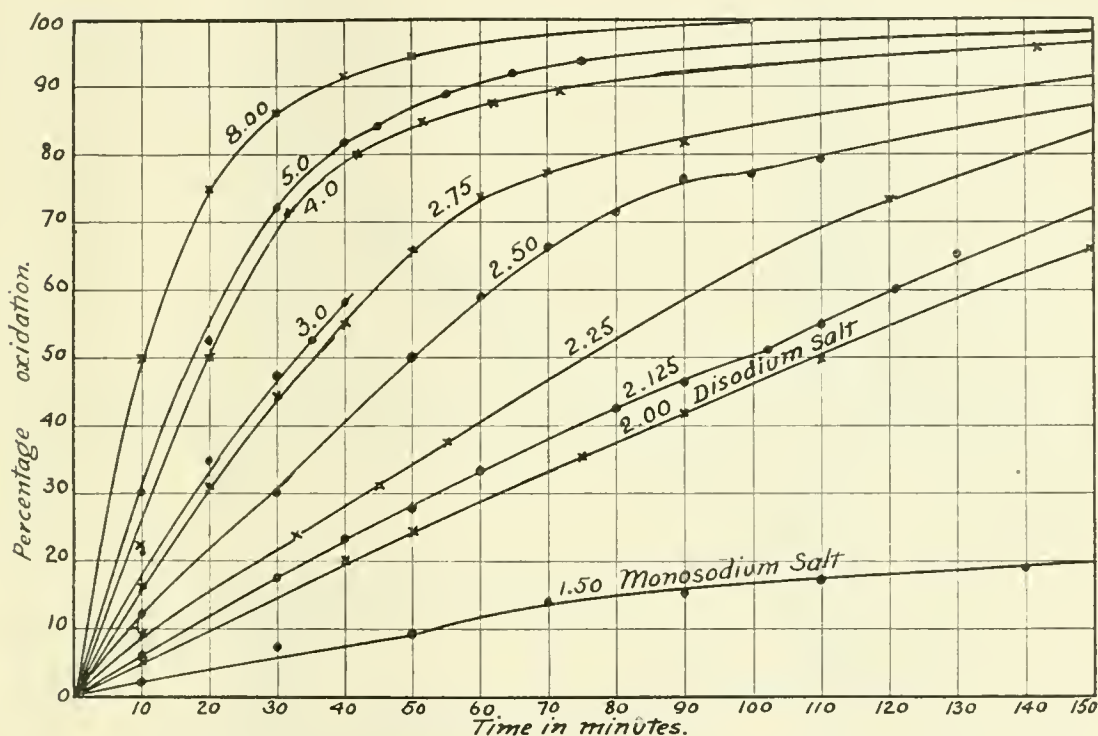


CHART 2. OXIDATION OF ARSPHENAMINE. .05 m/L

The figures on the curves give the concentrations of NaOH present in milliequivalents per liter. Note the uniform nature of the curves as contrasted with "arsenoxide," chart 1. Since two-thirds of the oxidation of arsphenamine consists of the oxidation of "arsenoxide" to the pentavalent form, the difference between the way the reaction proceeds here and with the pure "arsenoxide" is obvious.

of alkali. This relation can be seen at a glance at the data plotted in charts 2 and 3. In chart 3 the reciprocal of the time required for 50 per cent oxidation is plotted against the concentration of alkali. Since the concentration of arsphenamine hydrochloride is 0.5 m/L, 1.5 m/L NaOH are required to form the monosodium

salt, and 2 m/L NaOH to form the disodium salt. The disodium salt oxidizes rather slowly, one hundred and ten minutes being required for 50 per cent completion at 38° when constantly saturated with air.

A few experiments were performed with similar solutions exposed to air in a graduated cylinder and an Ehrlenmeyer flask, without constant aeration, conditions which simulate the circumstances of practical use. The oxidation proceeded with about

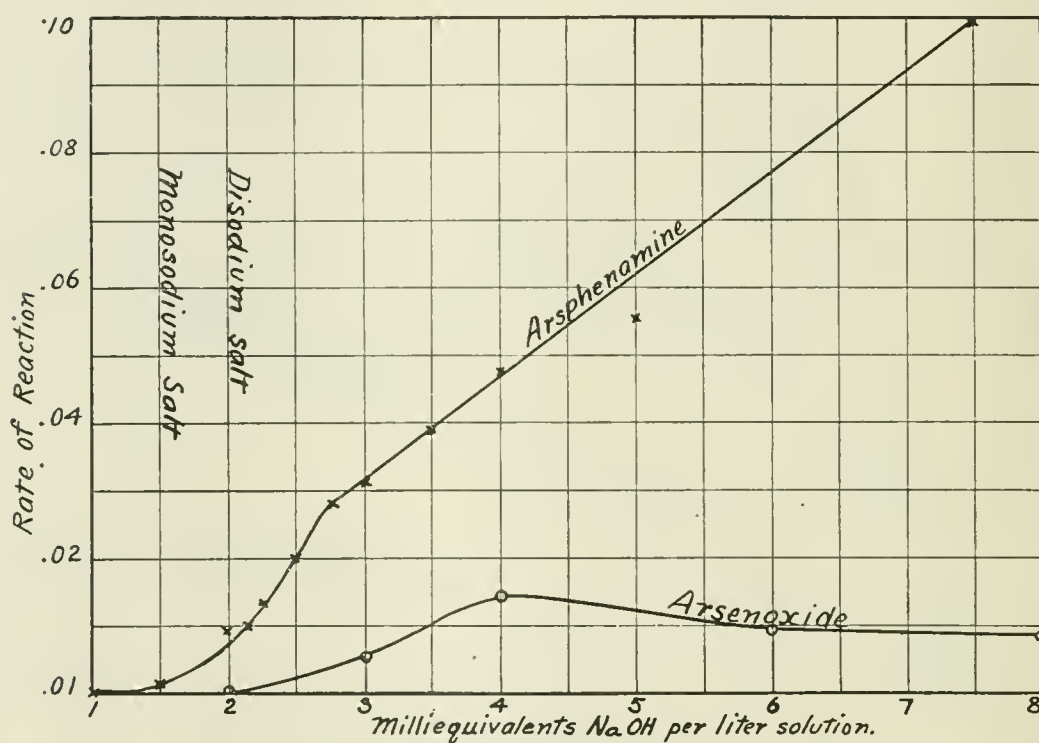


CHART 2a. RATE OF OXIDATION OF ARSPHENAMINE AND ARSENOXIDE

The reciprocal of the time required for the oxidation to proceed half way, as determined by interpolation, is plotted against the concentration of alkali present.

the same rapidity in both containers, and was nearly as rapid as when constantly aerated (table 2n). The extent of oxidation will of course depend upon the amount of aeration which the solution receives through shaking, the surface exposed, the excess of alkali present, and it may be greatly accelerated by the presence of positive catalysts. These experiments indicate that there is no great margin of safety, and that *such solutions should be used as soon after neutralization as possible.*

TABLE 2

(a) 0.05 m/L arsphenamine (dihydrochloride). No oxidation in 2 hours

(b) 0.05 m/L arsphenamine (dihydrochloride), 1 m/L NaOH. No oxidation in 1.5 hours

TIME IN MINUTES	PERCENTAGE OXIDATION
(c) 0.05 m/L arsphenamine (dihydrochloride), 1.5 m/L NaOH	
10	1.7
30	7.5
50	9.5
70	13.2
90	15.0
110	17.0
140	19.0
195	23.5
255	27.5
320	30.0
405	35.0
(d) 0.05 m/L arsphenamine (dihydrochloride), 2.00 m/L NaOH	
10	5.5
40	20.0
50	24.5
75	35.5
90	41.5
110	50.0
130	55.0
150	65.5
(e) 0.05 m/L arsphenamine (dihydrochloride), 2.125 m/L NaOH	
10	6.0
30	17.5
40	22.8
50	28.0
60	33.0
80	42.4
90	46.5
110	54.5
121	60.0
130	65.0

TABLE 2—*Continued*

TIME IN MINUTES	PERCENTAGE OXIDATION
(f) 0.05 m/L arsphenamine (dihydrochloride), 2.25 m/L NaOH	
10	0.9
21	23.0
33	24.0
45	31.0
55	37.5
120	73.0
(g) 0.05 m/L arsphenamine (dihydrochloride), 2.5 m/L NaOH	
10	12.0
20	24.0
30	30.0
50	50.0
60	59.0
70	66.0
80	71.0
90	76.0
100	77.0
110	79.0
(h) 0.05 m/L arsphenamine (dihydrochloride), 2.75 m/L NaOH	
10	17.0
20	31.0
30	44.5
40	54.5
50	65.8
60	73.6
70	77.0
90	81.6
(i) 0.05 m/L arsphenamine (dihydrochloride), 3.0 m/L NaOH	
10	16.0
20	34.5
30	47.0
35	53.0
40	58.0
(j) 0.05 m/L arsphenamine (dihydrochloride), 4 m/L NaOH	
10	22.0
32	71.6
42	80.0
52	84.2
62	87.5
72	89.2
142	95.9

TABLE 2—*Concluded*

TIME IN MINUTES		PERCENTAGE OXIDATION
(k) 0.05 m/L arsphenamine (dihydrochloride), 5 m/L NaOH		
10		30.0
20		52.0
30		72.0
41		80.0
45		83.7
55		88.5
65		91.5
75		93.3
(l) 0.05 m/L arsphenamine (dihydrochloride), 8 m/L NaOH		
10		50.0
20		75.0
30		85.9
40		91.3
50		94.5
100		±100.0
m/L NaOH	50 PER CENT CONVERSION TIME IN MINUTES	RATE OF REACTION
(m) Rate of oxidation of arsphenamine, at 38°		
0	∞	∞
1.00*	∞	∞
1.50†	±600	0.0016
2.00‡	110	0.0091
2.125	100	0.0100
2.25	75	0.0133
2.50	50	0.0200
2.75	35	0.0286
3.00	32	0.0310
3.50	26	0.0385
4.00	21	0.0475
5.00	18	0.0555
8.00	10	0.1000
TIMES IN MINUTES	100 CC. IN 200 CC. GRADUATED CYLINDER	100 CC. IN 200 CC. EHRLÉNMEYER FLASK
(n) 0.1 m/L arsphenamine (hydrochloride), 0.4 m/L NaOH (disodium salt)		
25	10.0	10.5
50	16.5	18.0
120	26.0	27.0
19 hours	70.0	73.4

* Suspension of precipitated base.

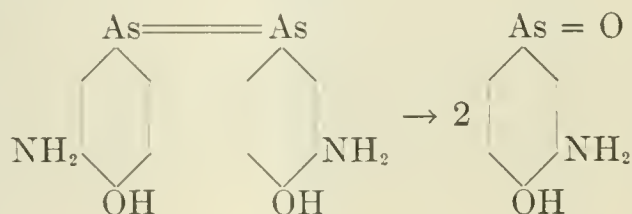
† Mono-sodium salt.

‡ Di-sodium salt.

An M/1000 solution of arspenamine dihydrochloride was boiled in an Ehrlenmeyer flask with a glass tube for a reflux condenser for an hour and fifteen minutes. The boiled solution was then diluted to the original volume and titrated. It showed 13.5 per cent oxidation. A similar experiment was repeated boiling for only fifteen minutes; this time there was only less than 1.0 per cent oxidation.

An M/1000 arspenamine dihydrochloride solution was placed in the oxidizing cylinder (38°) and constantly aerated for four days. It showed only 2 per cent oxidation in the first twenty-four hours, and 15 per cent oxidation in ninety-two hours. A slight precipitate formed during this time, presumably the carbonate which is insoluble in water, formed by some CO₂ passing the KOH wash bottle. Assuming that the entire decrease in titration value represents oxidation, it is evident that the dihydrochloride is extremely stable, for in this experiment *there was only 15 per cent oxidation in ninety-two hours under most favorable conditions for oxidation, i.e., constant saturation with air at body temperature.* These experiments lead us to believe that as the dry powder, arspenamine (the dihydrochloride!) may be considered as quite stable and not at all susceptible to oxidation, even when exposed to the air for long periods of time.

As each molecule of arspenamine is oxidized it must presumably pass through the oxide stage,



We have shown that this oxide is the trypanocidal factor, and that its trypanocidal activity is to be attributed to the $-\text{As}=\text{O}$ group.

It is therefore of great importance to know how this oxidation of the arseno-benzol compound, arspenamine, proceeds. Is it converted quantitatively to the oxide as the first step in the process, or is there a constant ratio of $\text{RAs}=\text{AsR}$ and $\text{RAs}=\text{O}$

maintained? In order to answer this question, it is necessary to determine not only the total trivalent arsenic present (such is the figure derived from the simple iodine titration), but also the concentration of "arsenoxide." This was done by a modification of the method of Ehrlich and Bertheim (2).

Two samples of the solution under investigation are taken. One is carefully measured, acidified with HCl and titrated with iodine. This titration figure represents the concentration of trivalent arsenic: As^{III} . The second sample is not a measured one, but must be larger than the desired quantity for titration. It is also acidified with a drop of concentrated HCl to stop further oxidation and when several such samples have been accumulated they are neutralized by the addition of 100 mgm. of NaHCO_3 . 200 mgm. of purified CaCO_3 are then added, the mixture well shaken, and filtered through a dry filter into a dry flask. The desired portion for titration is then withdrawn from the filtrate and titrated after the addition of several drops of HCl to decompose the excess NaHCO_3 . Most of the arsphenamine present in the original solution is precipitated by the addition of the NaHCO_3 , and the remainder as the insoluble calcium salt. The filtrate contains then only the oxide and the pentavalent arsenic. The arsphenamine content is determined by difference,

$$\text{RAs}^{\text{III}} - \text{RAs} : \text{O} = \text{RAs} : \text{AsR}.$$

The method is applicable only to fairly concentrated solutions (i.e., m/200), and is unsatisfactory with extremely dilute solutions, such as m/2000, where the precipitation of the arseno-benzol compound is incomplete.

By following the relative distribution of oxide and arsphenamine in the mixture of trivalent arsenic which remains at any one time, we see that the oxidation proceeds in a different manner at different rates of reaction (tables 3a-c, chart 3). In every case the relative concentration of oxide (column 3, table 3a-c) rises from the beginning to a maximum which varies with the rate of oxidation, then falls again as the reaction proceeds so that the last portion to oxidize is presumably 50 per cent oxide and 50 per cent arsphenamine. It will be seen that the more rapid the reaction the smaller is the percentage of oxide which

TABLE 3

TIME IN MINUTES	PERCENTAGE TOTAL OXIDATION	PERCENTAGE RAsO	PERCENTAGE RAs = AsR	PERCENTAGE TOTAL CONCENTRATION
(a) 5 m/L arsphenamine (dihydrochloride), 80 m/L NaOH (60 m/L NaOH in excess of disodium salt)				
0	3.0	6.2	93.8	6.2
10	16.0	13.2	86.8	11.1
20	29.0	13.6	86.4	9.6
30	43.0	15.2	84.8	8.7
40	54.0	17.9	82.1	8.2
50	64.3	17.5	82.5	6.2
60	74.4	17.2	82.7	4.8
70	82.2	18.9	81.1	3.4
90	90.4	35.0	65.0	3.4
110	94.2	50.0	50.0	2.9
130	96.1	37.5	62.5	1.5
(b) 5 m/L arsphenamine (dihydrochloride), 25 m/L NaOH (5 m/L NaOH in excess of disodium salt)				
0	3.7	5.8	94.2	5.8
10	13.6	11.6	88.4	10.0
25	32.1	20.9	79.1	14.2
40	46.8	32.3	67.7	17.3
55	56.8	48.7	51.3	21.0
70	65.3	60.7	39.3	21.0
90	72.1	70.0	30.0	19.5
110	76.9	77.2	23.8	17.9
130	80.6	75.6	24.4	14.7
180	85.3	67.8	32.2	10.0
200	86.7	64.0	36.0	8.4
240	89.5	60.0	40.0	6.3
270	92.1	53.0	47.0	4.2
(c) 5 m/L arsphenamine (hydrochloride), 20 m/L NaOH (disodium salt)				
0	5.0	10.3	89.7	10.3
10	12.8	18.3	81.7	16.0
25	27.3			
40	35.0	28.6	71.6	18.5
55	39.2	32.2	67.8	19.6
70	44.8	41.0	59.0	22.2
90	51.6	48.0	52.0	23.0
110	56.7	50.0	50.0	21.6
140	64.0	55.7	44.3	20.1
160	67.6	55.7	44.3	18.1
190	71.2	55.2	44.8	16.5

exists in the mixture at any one time and consequently we should not expect the absolute concentration of oxide to rise to as high a figure under these circumstances as when the reaction proceeds more slowly. We may indicate the absolute concentration of

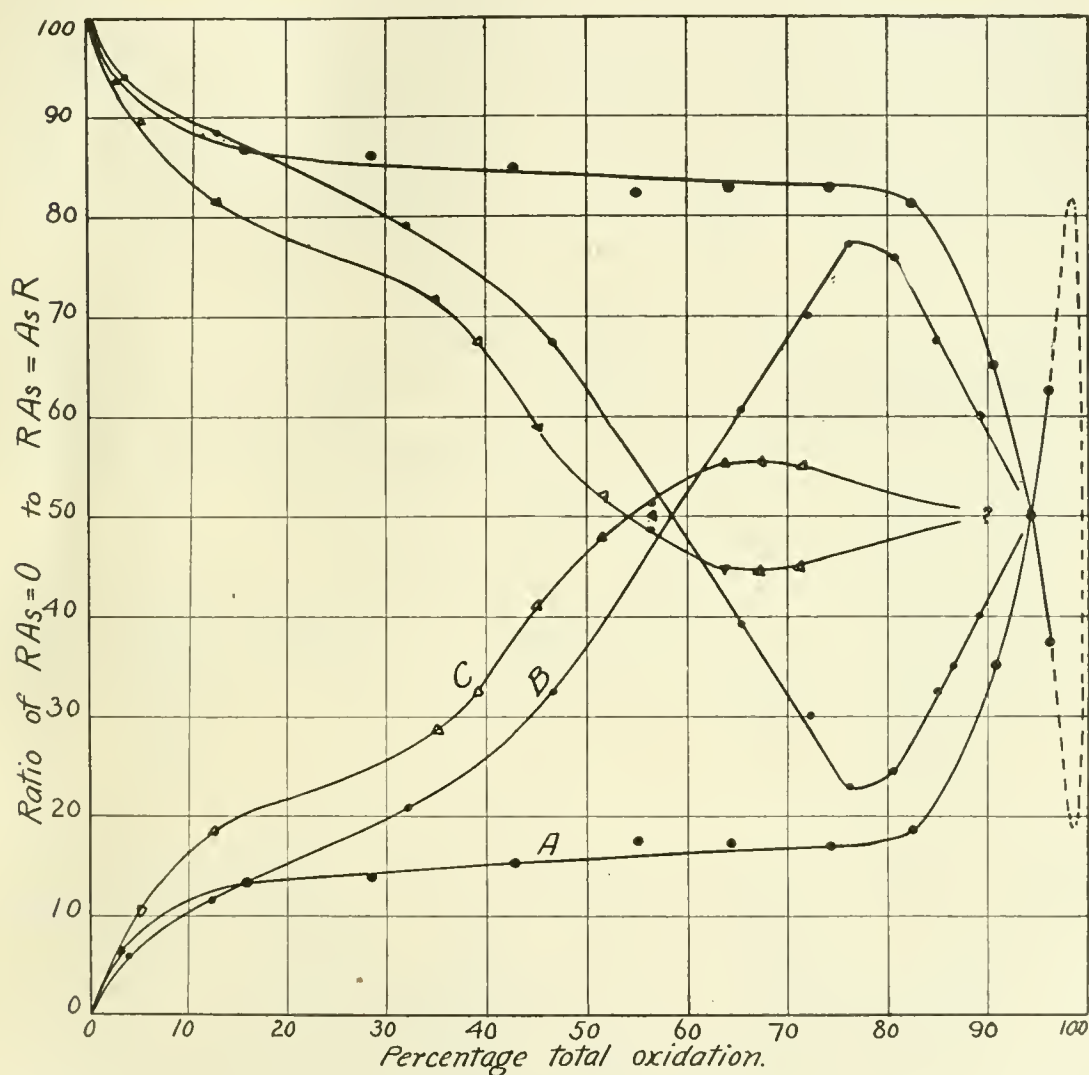


CHART 3. RATIO OF ARSENOXIDE AND ARSPHENAMINE DURING THE OXIDATION OF THE LATTER

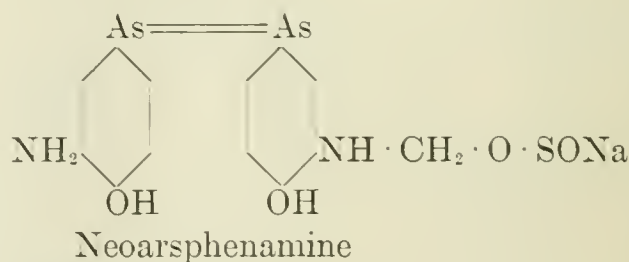
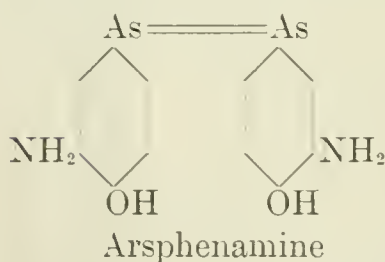
The letters on the curves refer to tables 4a, 2b, 4c, and show the relative distribution at different rates of reactions due to different hydroxyl-ion concentrations, as follows: A, 60 m/L NaOH in excess of disodium salt; B, 5 m/L in excess of disodium salt; C, no alkali in excess of disodium salt. Note: The upper curves ($RAs = AsR$) are of course the complements of the experimentally determined lower ones for $RAsO$. The extension of "A" beyond 94.2 per cent total oxidation is purely hypothetical and based upon the behavior of the other two curves.

arsenoxide in terms of the total trivalent arsenic present at the beginning of the experiment,

$$\text{Percentage concentration RAsO} = \frac{\text{RAsO}}{\text{RAs}^{\text{III}}}.$$

Expressed in this manner (see column 5, table 3a-c) it is evident that the greatest concentration of oxide is formed when the oxidation proceeds most slowly. In this connection it is interesting to note that Ehrlich (3) found a gradual increase in toxicity in arsphenamine solutions which were allowed to stand in contact with the air. This increase amounted to about 200 per cent in two hours and 350 per cent in six hours. The curve given by Ehrlich is essentially a straight line, and resembles the oxidation of the disodium salt (see chart 2). Similarly we have shown that the trypanocidal action of arsphenamine solutions increases rapidly when in contact with air, and the nature of the curves demonstrates the presence of "arsenoxide" (4).

Neoarsphenamine. This is a preparation of very uncertain composition. In its manufacture it is assumed that one (or sometimes both) amino group of arsphenamine is substituted by a sulphoxalate radical



The theoretical arsenic content in this substituted compound is 30 per cent, while that of the commercial product varies between 18 and 20 per cent. This wide discrepancy between the calculated and observed arsenic contents is largely due to contamination with sodium chloride, but there may also be present some free sodium sulfoxalate.

A few oxidation experiments were performed, but only a typical one will be reported. Since neoarsphenamine is a sodium salt, the addition of alkali is unnecessary. The solutions were made

in distilled water which had been boiled to expel oxygen, and poured while hot into a measuring flask which was nearly filled. The neoarsphenamine was added after cooling to 38° and dissolved with as little shaking as possible. The titration made immediately after the addition of the neoarsphenamine was used to indicate 100 per cent oxidizable material. There is a rapid fall in the iodine titration during the first five to ten minutes, indicating about 50 per cent oxidation; after this further oxidation takes place very slowly (table 4). The observations illustrated by table 4 were made with a lot of neoarsphenamine which

TABLE 4
0.05 m/L neosalvarsan

TIMES IN MINUTES	TITRATION, IODINE	PERCENTAGE OXIDATION
	<i>cc.</i>	
0	29.7	
5	19.9	33.0
10	19.5	34.5
20	18.3	38.5
30	15.4	48.0
40	14.0	53.0
50	13.0	56.2
70	12.4	58.5
90	11.8	60.3

should titrate only 19 cc. on the basis of its arsenic content. Since there is presumably one molecule of sulphoxalate in each molecule of neoarsphenamine, the sample should titrate 50 per cent too high, as each sulphoxalate group consumes three atoms of iodine. Sulphoxalate, however, is not oxidized by air, so the change in titration on aeration of the neoarsphenamine solution indicates oxidation of the arseno grouping.

A few experiments were performed with arsenophenylglycine which showed that this compound is rapidly decomposed in the same manner as neoarsphenamine. It is fairly certain that the arseno grouping in both neoarsphenamine and arsenophenylglycine is much more readily broken to form the oxide than in arsenamine, and that this decomposition is what we are actually

observing in the substitution of the amino group. Ehrlich found that neoarsphenamine solutions standing at room temperature in contact with air showed an increase of 350 per cent in toxicity in thirty minutes; after this the increase was very gradual. The nature of the curve which he gives is very much the same as the curve representing the oxidation, which shows a rapid decomposition at first, and then a gradual change. Roth (5) has recently found that shaking neoarsphenamine solutions with air for ten minutes increased the toxicity over 400 per cent, and in the previous paper we have given curves showing the increase in trypanocidal activity when such solutions are allowed to stand in contact with the air.

SUMMARY

1. The sodium salts of the following arsenicals are relatively very stable towards atmospheric oxygen: arsenious acid, methyl and ethyl arseniousoxide, phenyl and diphenylarsenious oxide, p-amino phenylarseniousoxide.

2. Arsphenamine (dihydrochloride) contrary to the prevalent views, is exceedingly stable towards atmospheric oxygen.

The addition of alkali leads to a rapid increase in the rate of oxidation of the compound, the rate of oxidation being roughly inversely proportionate to the hydroxyl ion concentration.

3. The sodium salt of arsphenamine is first oxidized to the corresponding oxide and this compound is simultaneously oxidized to the pentavalent arsenical.

The relative concentration of unchanged arsphenamine and oxide depends on the rate of reaction. The slower the reaction the more oxide is formed, but in every case the last portion to be oxidized consists of 50 per cent arsphenamine and 50 per cent oxide.

4. m-amino-p-oxyphenylarsenious oxide ("arsenoxide") is oxidized only in alkaline solution. The nature of the process curve indicates that the reaction is catalyzed by a reaction product.

5. Neoarsphenamine shows a rapid oxidation on exposure to air, amounting to about 50 per cent in the first ten minutes, after which the rate decreases rapidly.

6. The nature and rate of oxidation of arsphenamine and neo-arsphenamine to the corresponding oxides furnish an explanation of the increase in toxicity and trypanocidal activity of these compounds, when their solutions are exposed to air.

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EFFECT OF OPIUM ALKALOIDS ON THE BEHAVIOR OF RATS IN THE CIRCULAR MAZE

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The effect of drugs on psychological functions and animal behavior has been the subject of remarkably little investigation on the part of either psychologists or pharmacologists. With the exception of two substances—alcohol and caffeine—the contributions to this subject have until very recently been few and meager, so that the field of what may be termed “psychopharmacology” is virgin soil, full of possibilities (1). In connection with an extensive study of the effects of opium alkaloids, individually and in combination with each other, conducted by one of the authors, it was found desirable to analyze as far as possible the effect of those drugs on the psychological functions, and for this purpose an investigation was undertaken dealing with the effect of the opiates on the behavior of albino rats. This could be conveniently done by the use of the circular maze, with a camera lucida attachment introduced by Watson (2). Through the kindness of Prof. John D. Watson, the authors had the opportunity of conducting their experiments with the original apparatus designed by the former. This apparatus is described briefly as follows.

DESCRIPTION OF THE MAZE

The circular maze shown in figure 1 is made with wooden base and aluminium walls. The base is 150 cm. in diameter and 4 cm. in thickness. Its upper surface is marked off by grooves into a series of concentric circles. The diameter of each

of the circles is as follows, beginning with the outermost one: 140 cm., 120 cm., 100 cm., 80 cm., 60 cm., 40 cm., and 20 cm. Into the circular grooves are inserted sheets of aluminium 18.5 cm. high and 0.8 mm. thick. Each strip of aluminium is cut just 10 cm. shorter than the length of the circular groove into which it is to be fitted, thus giving an opening into the alley.

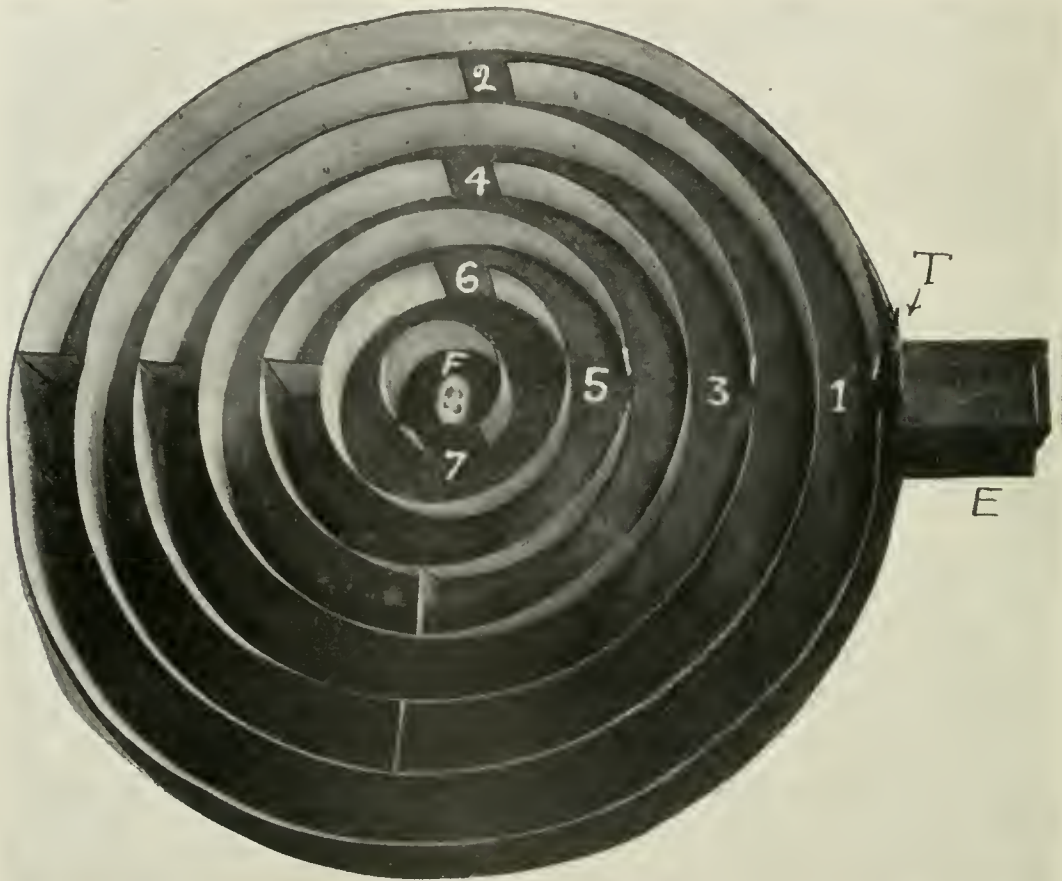


FIG. 1. CIRCULAR MAZE VIEWED FROM ABOVE

E, entrance cage; *T*, trap door leading into first alley; nos. 1 to 7 indicate the gates to the successive alleys; *F*, food.

By means of this arrangement it is possible to slide the aluminium around in its groove and thus to place the entrance in any desirable position. In the present investigation, the openings or entrances to the alleys were placed in the position indicated in figure 1, there being 7 openings so arranged that the rat had to make alternate turns to right and left, in the order indicated

by nos. 1 to 7. In addition to the doors or openings, the alleys are provided also with obstructing partitions, which form a

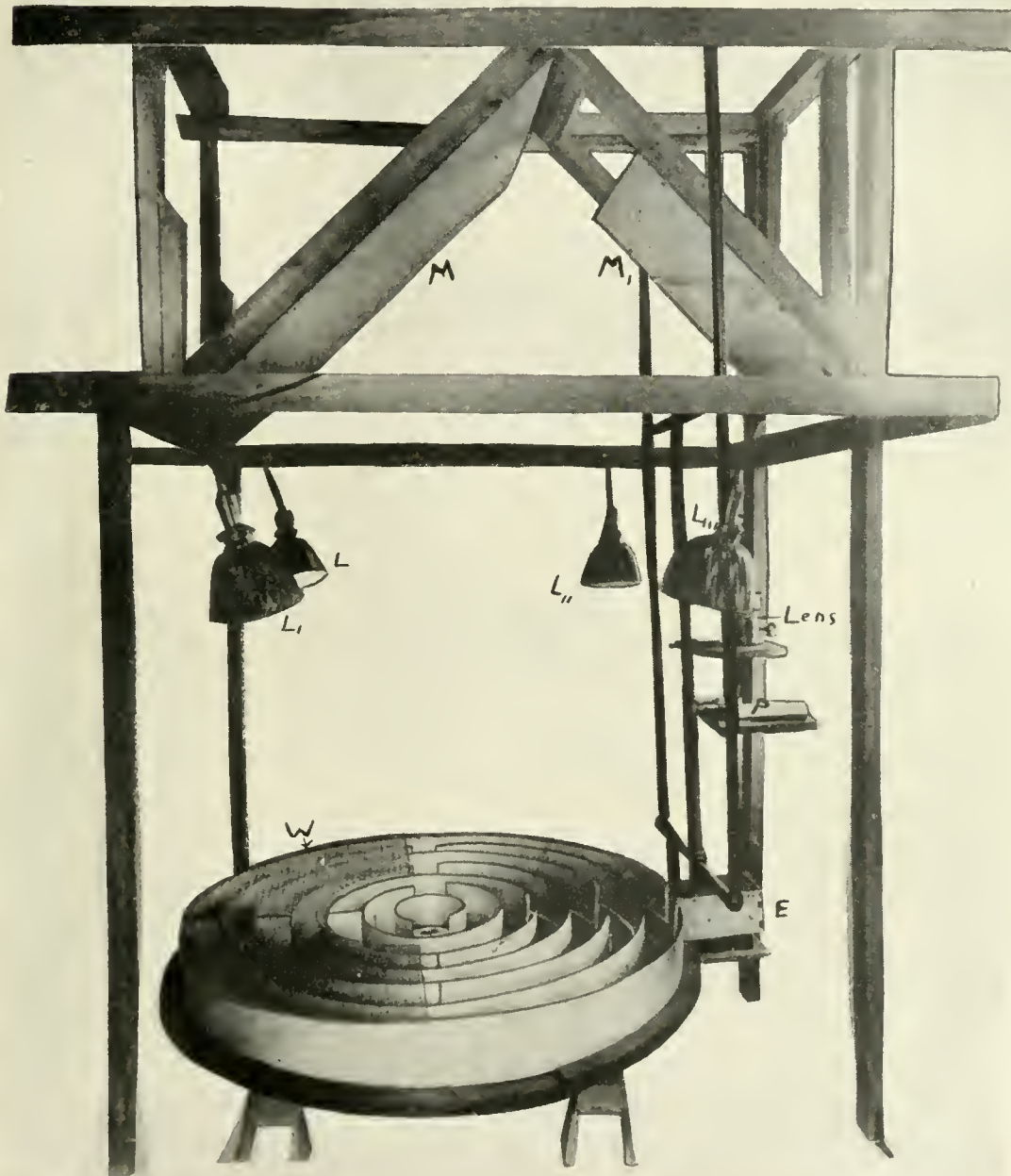


FIG. 2. CIRCULAR MAZE WITH CAMERA LUCIDA ATTACHMENT

Maze same as in figure 1. *W*, wire screen; *M* and *M*₁, reflecting mirrors; *L*, *L*₁, *L*₂ and *L*₃, electric lamps.

number of blind cul-de-sacs. A wire screen half of which is shown (*W*, fig. 2), prevents the animals from crawling over the top.

The camera lucida attachment invented by Professor Watson is illustrated in figure 2. A large plate-glass mirror M , 91 cm. wide and 121 cm. in length, was placed at an angle of 45 degrees, directly over the center of the maze. At a certain distance from this mirror a second mirror, M_1 , 60 cm. by 75 cm., is placed at an angle of 45 degrees above the maze, at such a distance from the first mirror that the light reflected downward from it falls outside of the maze. Below M_1 and in the light reflected from it, is placed a single achromat, 6 cm. in diameter and 50 cm. focus. The lens is placed in a barrel and the barrel is attached to a wooden disc 30 cm. in diameter. This board is attached to an iron collar which slides freely up and down and gives a very easy means of adjusting the size of the image. A circular piece of paper is laid upon a wooden shelf, below the lens, and the distance is so adjusted that the reduced image of the maze is focused upon the paper. Extraneous light is excluded by means of a soft dark flannel curtain, not shown in the figure. As may readily be seen from the figure, the maze must be illuminated quite highly in order to produce a clear image. This illumination is obtained by means of four powerful electric lamps, with opaque shades placed symmetrically around the maze. By means of the camera lucida attachment, the movements of an animal in the maze can be traced upon white paper with a soft pencil. Such tracings are especially useful in the study of the learning of the maze problem, such as has been done by Miss Hubbert and others. In the present investigation, where the effect of drugs on the behavior of the animals was studied after the rats had been trained, the use of this attachment was not essential and it was therefore dispensed with in a great many of the experiments.

The study of the behavior of the rats in the circular maze is begun by placing an animal in the center of the maze and feeding it from the bowl F for three successive days. During these three preliminary feedings, which last from ten to fifteen minutes, the entrance γ is blocked off, so that the animal may not roam around. On the fourth day, the rat is placed in the cage E , then the trap-door T is raised and the animal allowed to enter

the first alley. The animal then gradually learns to find its way to the center of the maze, when it is taken out and the experiment is repeated. Generally three trials are made on each day. For work with the maze, albino rats, which are very tame, must be employed. The animals must be handled gently with the hands and under no circumstances must they be picked up with forceps or similar instruments. The most suitable animals are found to be rats of approximately sixty to ninety days old. Older animals are apt to be sluggish, while very young rats do not learn the maze problem so readily. Ordinarily the albino rats learn the maze problem in about two weeks, and sometimes within a shorter period of time. An animal is considered to have solved the maze problem when it has learned to find its way into the center of the maze by the shortest route, that is, without any errors, on three successive trials. The technic of training is described more in detail by Hubbert (3).

ANALYSIS OF THE DATA FURNISHED BY THE MAZE

The maze problem enables the psychologist to study the mode of learning of a rat. In studying the effect of drugs, the maze problem can be utilized in two ways. Animals may be subjected to the influence of drug action first and then trained in the maze with the purpose of ascertaining the effect on the rate of learning. Again, animals may be first taught to solve the maze problem and then the effect of a drug is studied in reference to its influence on their behavior, memory-habit, etc. Furthermore, other data can be obtained from the maze, after administering drugs to rats, which may show the effect on neuromuscular coördination, and various somatic changes. As to exactly what the mechanism of learning the maze problem may be, the explanations given by various psychologists differ widely. Among the hypotheses which have been advanced to account for the reintegration of conduction paths in learning, there are at least three which stand out as rather opposed to one another in respect to the neural processes which they imply (4). The hypothesis suggested by Ladd and Woodworth (5) assumes inhibition of successive activi-

ties as the fundamental process which results in the selection and fixation of random activities. The second hypothesis, given by Angell and others (6), assumes nervous reinforcement as the fundamental process by which successive acts become linked together in habit-formation. The third hypothesis (Watson, (7)) depends chiefly upon the chance spreading of nervous excitation, or the simultaneous activation of two afferent pathways in such a way that the final common part of one is able to divert the discharge of the other and so bring about a permanent connection between itself and this afferent path. These hypotheses by no means exhaust the theoretical considerations of the maze problem (Dashiell (8)). For the study of drug action, however, the various theoretical considerations are of secondary importance and the data obtained are of a much more definite nature, as will be seen from the following exposition.

EXPERIMENTS WITH OPIUM ALKALOIDS

In the present research a total number of 24 rats were used. Most of these animals were from two to three months old and a few were between three and four months of age. The animals were fed on milk and bread once a day, receiving sufficient nourishment to maintain good health and gain slightly in weight, but not an excess of food, which generally renders these animals lazy. The rats were trained in the maze until they could solve the problem perfectly; that is, until they could run to the center of the maze by the shortest route in the shortest period of time. The period of time for that performance varied with the individual rats, the shortest times being four or five seconds. Many of the animals could run from the trap-door to the center of the maze without committing any errors, that is, by the shortest route possible within that period of time.

After the animals had been trained the effect of drugs was studied by injecting solutions of the same subcutaneously or intraperitoneally and the subsequent behavior of the animals was studied after a period of about thirty minutes. The points noted after administration of the drug were the time of perform-

ance, the number of errors or the total distance traversed by the animal, the general behavior, various somatic changes, if present, and the after effects. No animal was used again for a drug experiment until it had completely recovered its normal condition.

The following drugs were studied: Morphin, codein, papaverin, narcotin, narcein and thebain. After studying the individual drugs, the following combinations were employed: Pantopon, or a mixture of total opium alkaloids, containing 50 per cent of anhydrous morphin; and narcophin, a mixture of morphin and narcotin meconates, in the proportion of one to two.

THE EFFECT OF MORPHIN

The effect of injections of morphin sulphate in physiological saline solution was studied on 12 rats. Thirty-five experiments were performed in all. Most of the animals used had been trained by being placed in the maze three times in succession each day, until they learned to solve the problem perfectly. A few of the animals were trained by being placed in the maze only twice in succession each day. The doses of morphin administered varied from 0.2 mgm. to 10 mgm., each rat weighing on an average about 100 grams. While a dose of 10 mgm. may seem excessive, it is to be borne in mind that rats are not as susceptible to the toxic effects of morphin as higher animals, as has been shown by Macht (9) and others. The results of the morphin experiments are shown in table 1. It will be noted that the drug, in most of the experiments, produced a depression, as indicated either by the longer time of performance or by the number of errors made, or by both. In at least one animal, however, on repeated occasions, a small dose of morphin (0.5 mgm.), produced an excitation instead of a depression. This is very interesting to note, as a similar idiosyncrasy to morphin has been noted in respect to its analgesic properties (10).

After administration of morphin, in almost every experiment, the so-called Straub's biological reaction was noted (11). This, as is well known, consists of a peculiar stiffening or curling of the tail, and an analysis of this peculiar phenomenon has been made

TABLE I
Effect of morphin

NUMBER OF RAT	DOSE <i>mgm.</i>	NUMBER OF TRIALS		TIME OF PERFORMANCE		DISTANCE TRAVERSED IN METERS		EFFECT ON BEHAVIOR	SOMATIC EFFECT	AFTER EFFECTS
		Before drug	After drug	Before drug	After drug	Before drug	After drug			
I	10.0	3	Stalled	22"	Failure	13.5	Stalled	Does not move	Sleeping	Very marked
I	1.0	6	6	37"	47"	27.5	29.2	Sluggish	Straub's sign	None
I	0.5	3	3	35"	23"	15.7	13.5	Stimulation	Straub's sign	None
III	2.0	3	Stalled	32"	Failure	16.2	Stalled	Depression	Straub's sign	Slight
III	1.0	6	Stalled	58"	Failure	30.8	Stalled	Depression	Straub's sign	Slight
III	0.5	3	3	21"	31"	14.1	16.2	Depression	Straub's sign	Recovery next day
IV	2.0	3	3	18"	22"	13.5	13.5	Very little effect	Straub's sign	None
IV	1.0	6	5	32"	2' 19"	27.5	36.2	Depression	Straub's sign	None
IV	0.5	6	6	31"	2' 34"	28.5	38.4	Depression	Straub's sign	None
IV	0.2	3	3	17"	25"	14.0	13.5	Depression	Straub's sign	None
V	5.0	3	Stalled	23"	Failure	14.2	Stalled	Depression	Straub's sign	Marked
V	1.0	4	4	82"	2' 12"	20.0	17.4	Depression	Straub's sign	Marked
VI	0.5	2	2	15"	48"	9.0	11.7	Depression	Straub's sign	Slight
VII	1.0	2	2	18"	4'	9.2	21.4	Depression	Straub's sign	Slight
VII	0.5	4	4	1' 42"	5' 3"	24.8	35.5	Depression	Straub's sign	Slight
VIII	5.0	3	Stalled	19"	Failure	13.5	Stalled	Depression	Straub's sign	Marked
VIII	0.5	6	6	1' 53"	4' 49"	35.1	43.3	Depression	Straub's sign	Slight
IX	10.0	3	Stalled	15"	Failure	13.5	Stalled	Depression	Straub's sign	Very marked
IX	2.0	3	3	23"	31"	14.1	15.5	Slight depression	Straub's sign	None
IX	1.0	3	3	15"	15"	13.5	13.5	No effect	Straub's sign	None
IX	0.5	6	6	29"	49"	27.0	30.6	Slight retardation	Straub's sign	None
X	1.0	3	Stalled	16"	Failure	13.5	Stalled	Depression	Straub's sign	None
X	0.5	3	3	18"	42"	13.5	16.7	Depression	Straub's sign	None
X	0.2	3	3	30"	3' 21"	14.2	23.8	Depression	Straub's sign	Slight
XI	0.5	3	Stalled	21"	Failure	13.5	Stalled	Depression	Straub's sign	Marked
XI	0.2	3	Stalled	1' 13"	Failure	19.5	Stalled	Depression	Straub's sign	Marked
XII	0.5	3	Stalled	53"	Failure	17.6	Stalled	Depression	Straub's sign	Marked

by one of the authors recently, in which he showed that the rigidity of the tail is to be ascribed to the effect of the piperidin nucleus of the morphin molecule (12).

The authors have paid especial attention to the behavior of the animals on the days following injections of morphin. In some of the animals no after-effects were noted a few hours after injection of the drug; in others a depression was noted on the following day. After very large doses (10 mgm.), such a depression was noticeable for several days afterwards. It was interesting to note, however, that in no case was any animal permanently injured by morphin and that complete recovery followed sooner or later in every case.

ACTION OF OTHER OPIUM ALKALOIDS

The effect of injections of codein, papaverin, narcotin, narcein and thebain is summarized in table 2.

It was found that codein, in 9 experiments out of 11, produced a depression, and in 2 experiments, no change.

Narcotin, in 5 experiments out of 7, was depressant, and in 2 experiments produced little or no change.

Narcein, in 6 experiments out of 10, showed little or no effect, and in 4 experiments, a slight depression.

Thebain, in 1 experiment out of 11, showed a stimulation, and in 10 experiments, a retardation.

The least depressant of the individual alkaloids was found to be papaverin, which produced a depression only after very large or toxic doses, but otherwise had either no effect or showed a slight tendency to stimulation.

EFFECT OF COMBINATIONS

It was deemed desirable to investigate the effects of pantopon and narcophin, inasmuch as these substances have been found by Macht and others to produce synergistic effects, as compared with morphin, in respect to their action on the respiration (13), and in respect to their analgesic effects. The data obtained are shown in tables 3 and 4.

TABLE 2
Effect of papaverin, codein, narcotin, narcein and thebain

NUMBER OF RAT	DRUG INJECTED	DOSE	NUMBER OF TRIALS		TIME OF PERFORMANCE		DISTANCE TRAVERSED IN METERS		EFFECT ON BEHAVIOR	AFTER EFFECTS
			Before drug	After drug	Before drug	After drug	Before drug	After drug		
I	Papaverin hydrochloride	mgm. 10.0	3	3	26"	1' 22"	14.6	18.9	Depression	None
III	Papaverin hydrochloride	5.0	3	3	18"	20"	13.5	13.5	No effect	None
IV	Papaverin hydrochloride	5.0	3	3	27"	23"	13.5	13.5	No effect	None
VIII	Papaverin hydrochloride	5.0	3	3	19"	27"	13.5	14.1	No effect	None
IX	Papaverin hydrochloride	15.0	3	Stalled	18"	Failed	13.5	Stalled	Depression	Marked
II	Papaverin hydrochloride	15.0	3	Stalled	19"	Failed	14.0	Stalled	Great depression	Died
XI	Papaverin hydrochloride	5.0	3	3	28"	27"	14.1	14.0	Stimulation	None
XII	Papaverin hydrochloride	5.0	3	Stalled	1' 50"	Failed	20.6	Stalled	Depression	Memory bad
IX	Papaverin hydrochloride	5.0	3	3	23"	23"	13.5	13.5	No effect	None

I	Codein phosphate	2.0	3	3	19"	1' 43"	13.5	15.8	Depression	Slight
III	Codein phosphate	2.0	6	6	36"	4'	27.2	48.8	Depression	Slight
IV	Codein phosphate	2.0	3	3	15"	25"	27.0	29.2	Slight depression	None
VII	Codein phosphate	2.0	4	4	48"	1' 50"	20.0	23.2	Slight depression	None
VIII	Codein phosphate	2.0	3	3	28"	1' 20"	14.6	20.1	Depression	None
IX	Codein phosphate	2.0	3	3	21"	24"	14.1	14.5	No effect	None
IX	Codein phosphate	0.5	3	3	20"	19"	14.0	13.5	No effect	None
X	Codein phosphate	1.0	3	3	44"	2' 15"	17.1	20.8	Depression	Slight
XI	Codein phosphate	2.0	3	3	24"	27"	13.5	13.5	No effect	None

IV	Narcotin hydrochloride	2.0	3	3	15"	19"	13.5	13.5	No effect	None
VI	Narcotin hydrochloride	2.0	2	2	19"	24"	9.6	9.5	No effect	None
VIII	Narcotin hydrochloride	2.0	3	3	16"	32"	13.5	15.2	Slight depression	None
IX	Narcotin hydrochloride	2.0	3	3	21"	1' 24"	13.5	18.8	Slight depression	None
X	Narcotin hydrochloride	2.0	6	6	1'	2' 16"	9.6	37.8	Depression	Marked
XII	Narcotin hydrochloride	2.0	3	3	1' 15"	1' 15"	13.5	15.2	Depression	Slight

IX	Narcotin hydrochloride	3.0	3	3	15"	21"	13.5	14.1	No effect	None
XI	Narcotin hydrochloride	3.0	3	3	1' 50"	2' 30"	20.5	21.2	Slight depression	None
XVI	Narcotin hydrochloride	1.0	3	3	23"	2' 30"	13.5	20.8	Depression	Slight
XVII	Narcotin hydrochloride	1.0	3	3	37"	36"	15.6	14.0	No effect	Slight
XVIII	Narcotin hydrochloride	0.1	3	3	48"	30"	27.0	19.6	No effect	None
XIX	Narcotin hydrochloride	1.0	3	3	15"	1' 3"	13.5	36.0	No effect	None

TABLE 2 - Continued

NUMBER OF RAT	DRUG INJECTED	DOSE <i>mgm.</i>	NUMBER OF TRIALS		TIME OF PERFORMANCE		DISTANCE TRAVERSED IN METERS		EFFECT ON BEHAVIOR	AFTER EFFECTS
			Before drug	After drug	Before drug	After drug	Before drug	After drug		
XXII	Narcein hydrochloride	1.0	3	3	17"	23"	13.5	13.5	No effect	None
XXIII	Narcein hydrochloride	1.0	3	3	57"	59"	31.1	29.8	No effect	None
XXIII	Narcein hydrochloride	0.1	3	3	27"	24"	15.2	13.5	No effect	None
XXIV	Narcein hydrochloride	0.2	3	3	46"	50"	27.0	22.8	Depression	Slight

XII	Thebain hydrochloride	1.0	3	1	15"	39"	6.0	27.0	Depression	Marked
XVII	Thebain hydrochloride	1.0	3	Stalled	43"	Failed	29.5	Stalled	Depression	Marked
XVII	Thebain hydrochloride	0.2	3	3	26"	45"	14.0	27.5	Depression	Marked
XVIII	Thebain hydrochloride	1.0	3	Stalled	45"	Failed	35.7	Stalled	Depression	Marked
XIX	Thebain hydrochloride	1.0	3	Stalled	17"	Failed	13.5	Stalled	Depression	Marked
XIX	Thebain hydrochloride	0.1	3	2	15"	42"	13.5	28.2	Depression	Marked
XXII	Thebain hydrochloride	1.0	3	3	40"	32"	19.0	15.5	Stimulation	None
XXII	Thebain hydrochloride	1.0	3	Stalled	52"	Failed	34.0	Stalled	Depression	Slight
XXIII	Thebain hydrochloride	0.1	3	3	19"	45	13.5	27.0	Depression	Slight
XXIV	Thebain hydrochloride	0.2	3	3	21"	1' 7"	13.5	31.1	Depression	Slight
XXIV	Thebain hydrochloride	0.1	3	3	18"	54"	13.5	28.0	Depression	Slight

TABLE 3
Effect of pantopon

NUMBER OF RAT	DOSE mgm.	NUMBER OF TRIALS		TIME OF PERFORMANCE		DISTANCE TRAVERSED IN METERS		EFFECT ON BEHAVIOR	SOMATIC EFFECT	AFTER EFFECTS
		Before drug	After drug	Before drug	After drug	Before drug	After drug			
I	2	6	4	36"	94"	27.5	24.3	Depression	Straub present	Slight
I	1	3	3	23"	25"	13.5	14.1	Little effect	Straub present	None
II	2	6	6	38"	2' 47"	27.5	43.2	Depression	Straub present	Slight
III	2	3	3	19"	15"	14.0	13.5	No effect	No Straub	None
III	1	3	3	21"	25"	14.0	14.0	No effect	Straub present	None
IV	2	6	6	41"	55"	27.0	29.8	Slight depression	Straub present	None
IV	1	6	6	49"	41"	29.3	28.0	No effect	Straub slight	None
V	10	3	Stalled	30"	Failure	15.2	Stalled	Great depression	Straub absent	Marked
VI	2	4	4	30"	41"	18.6	30.4	Depression	Straub slight	Slight
VI	1	2	2	22"	28"	9.6	9.8	Little effect	Straub slight	Slight
VII	2	2	2	32"	90"	10.1	17.0	Depression	Straub present	Slight
VII	1	4	4	76"	2' 15"	28.5	34.6	Depression	Straub present	Slight
VIII	10	3	Stalled	31"	Failure	16.2	Stalled	Great depression	Straub absent	Marked
VIII	4	3	3	52"	3' 40"	17.0	27.8	Depression	Straub slight	Slight
VIII	1	3	3	33"	2' 53"	14.0	25.0	Depression	Straub slight	Slight
IX	4	3	3	15"	32"	13.5	15.6	Slight depression	Straub slight	None
IX	2	3	3	14"	17"	13.5	13.5	No effect	Straub slight	None
IX	1	6	6	31"	33"	27.0	27.0	No effect	Straub slight	None
X	4	3	3	70"	4'	18.1	27.2	Depression	Straub slight	Marked
X	2	3	3	19"	1' 23"	14.0	18.9	Depression	Straub slight	Slight
X	1	6	6	38"	1' 58"	27.0	32.5	Depression	Straub slight	Slight
XI	2	3	Stalled	1' 17"	Failure	18.1	Stalled	Depression	Straub absent	Marked
XI	1	3	Stalled	33"	Failure	14.6	Stalled	Depression	Straub absent	Marked
XII	2	3	Stalled	2' 40"	Failure	23.2	Stalled	Depression	Straub present	Marked
XII	1	3	3	30"	1' 45"	14.5	19.5	Depression	Straub present	Slight

TABLE 4
Effect of narcophen

NUMBER OF RAT	DOSE <i>mgm.</i>	NUMBER OF TRIALS		TIME OF PERFORMANCE		DISTANCE TRAVERSED IN METERS		EFFECT ON BEHAVIOR	AUTOMATIC EFFECT	AFTER EFFECTS
		Before drug	After drug	Before drug	After drug	Before drug	After drug			
I	3	3	3	45"	59"	17.8	18.5	Slight depression	Straub present	None
III	3	3	3	21"	60"	13.5	18.5	Depression	Straub present	None
IV	3	6	6	30"	32"	27.0	27.5	No effect	Straub present	None
VI	3	2	2	20"	2' 30"	9.6	18.8	Depression	Straub present	None
VII	3	2	2	36"	2' 5"	10.2	17.5	Depression	Straub present	None
VIII	3	3	3	1' 19"	5' 5"	19.3	36.9	Depression	Straub present	Marked
IX	3	6	6	31"	30"	27.0	27.0	No effect	Straub present	None
X	3	3	Stalled	48"	Failure	37.3	Stalled	Depression	Straub present	Marked
XI	3	3	3	36"	5' 22"	16.8	33.8	Depression	Straub present	Marked

A comparison of the data obtained from the same animals with pantopon or narcophin on the one hand and equivalent doses of morphin on the other (pantopon containing 50 per cent of morphin and narcophin containing 33 per cent of morphin), gave the following interesting results.

Out of 26 experiments, in which morphin and pantopon were given to the same animals, in 4 experiments there was an evidence of pantopon being more depressant than morphin, both in respect to the time of performance and the distance traversed. In 15 experiments, the effect of morphin was decidedly more depressant than that of pantopon, both in respect to the time of performance and the distance traversed. In 3 experiments, after large doses, the effects of morphin and pantopon were equivalent. In 1 experiment morphin was more depressant than pantopon in respect to the time of performance, but less depressant in respect to the number of errors made. In 3 experiments, pantopon was more depressant than morphin in respect to time, but morphin was much more depressant than pantopon in respect to the number of errors made.

On comparing the effect of narcophin with that of morphin, it was found that in 4 experiments morphin was more depressant than narcophin in respect to both the time of performance and the number of errors made; and in 3 experiments the effects of the two drugs were the same. In 1 experiment morphin was more depressant than narcophin in respect to time, but less depressant in respect to the number of errors. In 1 experiment narcophin was more depressant than morphin in respect to time, but less depressant in respect to the number of errors.

DISCUSSION

A careful study of all the data obtained showed that all of the opium alkaloids, with the exception of papaverin, exert a depressant effect upon the behavior of albino rats, even when given in comparatively small doses. This was indicated by impairment of the memory-habit and the activity of the animals. It was noted that the time of running through the maze was

prolonged and the number of errors committed was increased, and in many cases both the time and the distance traversed (= no errors made) were remarkably affected. Of the individual alkaloids, morphin was by far the most depressant.

It was interesting to note that in at least one case morphin produced a stimulating effect, the animal showing an idiosyncrasy for the drug, such as has been noted in connection with the effects of morphin on other physiological functions.

Straub's phenomenon was present in almost every case in which morphin was administered, but was exhibited though not so strikingly after other opium alkaloids of the morphin group (codein and thebain). After combinations, Straub's phenomenon was also present but not in such a high degree. The authors never noted this phenomenon after injections of papaverin, narcotin or narcein.

A comparison of the effects of pantopon and narcophin, on the one hand, and equivalent doses of morphin in the same rat, on the other hand, leads to the conclusion that morphin is, on the whole, more depressant than the above-named combinations.

Lastly, it was very interesting to find that in all experiments, even where large doses of the narcotics were administered and produced marked and prolonged depression or impairment of the cerebral and neuro-muscular functions, the animals sooner or later all recovered their normal behavior. This, of course, is encouraging experimental evidence in connection with the clinical treatment of the opium and morphin habit.

SUMMARY

1. The behavior of albino rats before and after injections of opiates, was studied in the circular maze.

2. It was found that morphin produced a depressant effect on the behavior of the animal, after both large and small doses, but that in the case of one animal the drug exhibited a stimulating effect.

3. Of the other opium alkaloids, with the exception of papaverin, all were found to be slightly depressant.

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Page 234. Third line from the top, instead of "no errors made" should read
'no. of errors made.'

4. Combinations of morphin with other opium alkaloids are, on the whole, somewhat less depressant than equivalent doses of morphin when given alone.

5. In all the experiments, even where large doses of the narcotics were administered, and marked and prolonged impairment of the psychological functions was produced, the animals eventually recovered their activity.

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A COMPARISON OF THE EFFECT OF CERTAIN SAPONINS ON THE SURFACE TENSION OF WATER WITH THEIR HEMOLYTIC POWER¹

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Saponins affect the surface tension of water in such a manner that their solutions may be made to foam; and therefore they have been used quite freely in certain foods and beverages in order to produce a foam or give the product the capacity to foam. They have, for example, been substituted in part for white of eggs in certain bakery products. Even though it is stated that many of the saponins are not readily absorbed from the gastro-intestinal tract, it is important to study their behavior both within and without the animal body, since, when introduced into the blood stream, they have the power of laking red blood corpuscles. Such studies may furnish information which will explain the mechanism of the physiological action of the saponins or lead to a method for their determination or identification more satisfactory than any now in use.

The investigation herein reported is such a study. It seeks to determine whether or not there is any such correlation between the surface tension of aqueous saponin solutions and their hemolytic activity as has been reported in the literature for the solutions of a number of other substances. Thus Czapek (1) found that exosmosis was caused in certain plant cells by water solutions of alcohols, acids and esters, as well as by emulsions of lecithin,

¹ Cf. H. E. Woodward and C. L. Alsberg.—The Relation of the Surface Tension of Saponin Solutions to their Hemolytic Activity. Scientific Proceedings of the American Society for Pharmacology and Experimental Therapeutics. Seventh Annual Session. The Journal of Pharmacology and Experimental Therapeutics, 1916, viii, 109.

cholesterol and fats when the surface tension of the solution was less than 69 per cent of the surface tension of water. According to Traube (2) solutions of hemolytic substances hemolyze only when the surface tension is below a certain value, and solutions with the same surface tension have the same hemolytic activity. His experimental work was done on aliphatic alcohols and esters. In an investigation of eleven terpene alcohols and ketones, Ishizaka (3) found for ten of them that the surface tension of the concentrations just sufficient to cause hemolysis was 81 per cent of that of water.

THE SAPONINS USED IN THIS INVESTIGATION

Twelve saponins were examined in this investigation. Seven of these were prepared very carefully in the Bureau of Chemistry incidental to chemical investigations on saponins from various plants. One was prepared from *Chlorogalum pomeridianum* by Dr. C. O. Johns; another from *Yucca radiosa* by Dr. Geo. A. Geiger; and others from *Yucca Angustifolia*, *Y. filamentosa*, *Chamelirium luteum*, *Agave lecheguilla*, and *Trillium erectum*, L. by Dr. L. H. Chernoff. Five saponins, digitonin, guajac, quillaja, sapindus and solanin preparations, were purchased from Merck.

The general method of the preparation of the saponins produced in the Bureau of Chemistry was published (4) subsequent to the completion of this investigation. For the purposes of this investigation it was sufficient to purify them until free from ash. They were used as received except the *Yucca radiosa* saponin, which was fractionated into two parts, one of which was more soluble in absolute alcohol than the other. The more soluble part was separated by precipitation with anhydrous ether. Of the *Agava lecheguilla* saponin two samples were used, prepared in different ways; by water extraction and by alcohol extraction respectively of the original plant material. The difference in the properties of the two preparations was probably due to the fact that the saponin obtained by alcohol extraction is decomposed in water solution. All the preparations were

dried in a vacuum oven at 70°C. and kept in stoppered bottles in a desiccator.

The *Chamelirium luteum* saponin probably differs from the others in that as prepared it may have been combined or contaminated with phytosterol or sitosterol. When a few milligrams were treated at room temperature with a small amount of acetic anhydride and two drops of concentrated sulphuric acid, it gave a green color like that given by cholesterol. Other saponins when treated in this way gave no color or only a light brown. However, *Chamelirium* saponin was soluble in Locke's solution to the extent of at least 1 per cent and chloroform did not extract any cholesterol or phytosterol from the dry saponin at room temperature.

All of the saponins obtained from Merck were used as purchased except the quillaja saponin which was further purified by two different methods. In the purification of the quillaja saponin by the first method it was dissolved in hot absolute ethyl or methyl alcohol from which it was separated either by cooling or by precipitation with anhydrous ether. Whether ethyl or methyl alcohol was used, whether the separation was effected by cooling or by the addition of ether, the products obtained seemed to differ but slightly from one another. In the second method purification was effected by heating a concentrated aqueous solution of quillaja saponin with saturated barium hydroxid, separating the resulting precipitate by filtration and decomposing this precipitate with carbon dioxid. The solution so obtained was then clarified by filtration and evaporated to dryness on a steam bath. The residue was dissolved in absolute alcohol and the alcoholic solution precipitated with anhydrous ether.

THE METHOD OF MEASURING SURFACE TENSION

The values for surface tension were determined by the drop weight method of Morgan (5). The tip of the apparatus was standardized with distilled water, with which it gave a drop weight of 67.986 mgm. at 37°C. Since the surface tension of water at 37°C. is 69.844 (6) the factor for converting the drop

weight in milligrams obtained with the apparatus used in the present investigation to surface tension in dynes per centimeter is 1.027. The surface tension determinations were made at 37°C. as that was the temperature used for the hemolytic tests. Five drops of the saponin solutions were taken from the tip at that temperature and weighed.

The determinations of the relative effect of the saponins on surface tension were made on solutions containing 100 mgm. of saponin per liter of Locke solution² except the determinations given in table 1 for which water was used. This concentration was chosen because, as described later in this paper, higher concentrations do not lower the surface tension much further. Locke solution was used instead of pure water because it was employed in the hemolysis tests. The solutions studied were made by dissolving 100 mgm. of the saponin in 1 deciliter of Locke solution and diluting 10 milliliters of this to 1 deciliter with Locke solution. The surface tension measurements and the hemolytic measurements were both made with the same solutions within two days of the time they were prepared. After standing a few days, the dilute solutions change slightly, and some of the solutions containing 0.1 per cent of saponin change within a few hours. The change is shown in some cases by development of a precipitate, and in others only by the change in the surface tension.

THE METHOD OF DETERMINING HEMOLYTIC ACTIVITY

The relative hemolytic activity of the saponins was found by determining the lowest concentration of the saponin which caused complete hemolysis, within two hours, of two drops of rabbit erythrocytes in 10 milliliters of Locke solution at 37°C. The rabbit erythrocytes were obtained by taking about 10 milliliters

² The composition of the Locke solution used was as follows:

	<i>Grams per liter</i>
NaCl.. . . .	9.2
KCl.	0.42
CaCl ₂	0.26
<u>NaKCO₃...</u>	0.15

2nd time slip

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Vol. XVI, no. 3, p. 240, last line: NaKCO_3 should read NaHCO_3 .

of blood from the hearts of rabbits, kept on a uniform diet of oats and hay, into about 50 milliliters of 0.9 per cent sodium chloride solution containing a little potassium oxalate, and centrifuging. The sediment of red corpuscles was then washed in a centrifuge with 0.9 per cent sodium chloride solution and then with Locke solution and was finally centrifuged in a narrow tube.

The solutions used for determining the hemolytic activity of the saponins were those used for the surface tension measurements. Different amounts of the solutions containing 100 or 1000 mgm. of saponin per liter were diluted in test tubes to 10 milliliters with Locke solution, and the tubes were placed in the constant temperature bath at 37°C. Two drops of the rabbit erythrocytes were then added, and the contents of the tube mixed by inverting. With the higher concentrations of saponin, the hemolysis was complete within a few minutes; but with the lower concentrations about one hour was required for completion of the reaction.

COMPARISON OF SURFACE TENSION EFFECT WITH HEMOLYTIC POWER

Surface tensions were determined for different solutions of some of the saponins. With increasing concentration of the saponin, the surface tension decreases rapidly up to a concentration of about 100 mgm. per liter, but with higher concentrations the surface tension is not lowered much further. The values found for solutions of chlorogalum and quillaja saponin of different concentrations are given in table 1. These solutions were made with water instead of Locke solution.

As above stated the determinations of surface tension were made upon solutions containing 100 mgm. per liter, of Locke solution. The results obtained are given in table 2, arranged in the order of the surface tension activity of the saponins. The values are the averages of two or more determinations. These values are not strictly comparable since the solutions were made up with equal weights instead of equivalent weights. The

molecular weights of the saponins were not known at the time. Certain of them have since been determined in the Bureau of Chemistry, and in one case the results have been published (7).

TABLE 1
*Surface tensions of aqueous saponin solutions of varyin gconcentration at 37°C.
Surface tension of water is 69.8 dynes per centimeter*

SAPONIN PER LITER	CHLOROGALUM	QUILLAJA
<i>mgm.</i>		
10	66.4	66.5
25	62.1	63.3
50	57.3	59.5
100	52.0	56.6
500	49.2	
1000	48.4	52.8

TABLE 2
*Surface tension at 37°C. of solutions containing 100 milligrams saponin per liter of
Locke solution*

	DYNES PER CENTI- METER
Digitonin	54.0
Chlorogalum.. . . .	55.4
Guiaç.....	55.5
Yucca angustifolia.....	56.6
Trillium.....	56.8
Yucca filamentosa.....	57.0
Quillaja from alcohol-ether.. . . .	57.2
Quillaja from alcohol.....	57.8
Chamelirium.. . . .	57.8
Sapindus....	58.1
Agave (by alcohol)....	59.5
Quillaja (by barium hydroxid).....	60.0
Yucca radiosa (from alcohol).. . . .	60.4
Solanin-HCl.....	62.5
Agave (by water).....	62.9
Yucca radiosa (from alcohol-ether)	63.1
Locke solution	70.1

However, examination of the unpublished data leads to the conclusion that there cannot be enough difference in the molecular weights to change the order of table 2.

The pH of the various solutions was not determined, but the data represent determinations made at different times with various lots of solution. The Locke solution was the same in all cases, and the saponins were of a high degree of purity. Moreover, since all the saponins have large molecules and the quantities used in these experiments were small, it is believed that any difference in pH of the various solutions would be so minute as to be negligible.

TABLE 3

Concentration of saponin causing hemolysis of two drops of rabbit erythrocytes in 10 millimeters, Locke solution at 37°C.

	SAPONIN MILLI-GRAMS PER LITER
Digitonin.	7
Sapindus.....	10
Chlorogalum.. . . .	10*
Quillaja from alcohol....	15
Quillaja from alcohol-ether..	15
Trillium.....	30
Quillaja (by barium hydroxid).....	40
Yucca angustifolia.....	40
Yucca filamentosa.....	50
Agave (by alcohol)....	80
Agave (by water).....	200
Yucca radiosa (from alcohol-ether).....	350
Solanin-HCl.....	600
Yucca radiosa (from alcohol).....	1,500
Guaiac.....	1,500*
Chamelirium—no hemolysis at.....	10,000

* Agglutination.

The results obtained in determining the hemolytic power of the saponin preparations are given below in table 3, arranged in the order of their power.

In table 3 the lowest concentration of chlorogalum and guaiac saponin causing complete hemolysis is not given because it could not be determined inasmuch as these saponins agglutinate the red corpuscles before they hemolyze them. However, these saponins seem to cause complete hemolysis at about three or four times the lowest concentration causing agglutination.

DISCUSSION

The data given in tables 2 and 3 do not show any relation between the hemolytic activity of the saponins studied and their effect on the surface tension of water. There is evidently no threshold value of surface tension below which hemolysis occurs and above which it does not occur. These data are not necessarily at variance with the conclusions arrived at by Traube, Czapek, or Ishizaka. The saponin solutions used in this investigation showed in all cases less than 25 per cent lowering of the surface tension. The substances which have been found by Traube, Czapek, and Ishizaka to show a relation between their hemolytic activity and the lowering of the surface tension are all substances which are soluble in lipoids. Probably all these phenomena, lipoid solubility, power to hemolyze, power to affect the surface tension are inter-related (8, 9, 10). The data herein presented indicate that the mechanism of saponin hemolysis is different. Possibly the mechanism may be dependent upon the property of many saponins to combine with cholesterol. For example it may be that saponins hemolyze because they attack the cholesterol of erythrocytes. In this connection it may be significant that the chamelirium saponin preparation examined in this investigation showed indications of the presence of cholesterol and did not hemolyze even in 1 per cent solution. At any rate the possibility of such a relationship is deserving of further investigation.

SUMMARY

The lowering of the surface tension of Locke solution by twelve saponins was measured.

The lowest concentration at which these saponins hemolyze was determined.

The surface tension effect and the hemolytic power of these saponins are not parallel.

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THE ACTION OF ADRENALIN ON THE HEART

III. THE MODIFICATION OF THE ACTION OF ADRENALIN BY CHLOROFORM

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Oliver and Schafer (1) in their original communication make note of an experiment in which the animal, a dog under the influence of chloroform, morphine, and atropine died suddenly following the injection of adrenal extract. They did not, however, advance an explanation for the phenomenon. Elliott (2) found that dogs under the influence of ether despite section of the vagi died suddenly due to heart failure with injection of adrenalin. He found large clots in the innominate vein and right heart and since death did not occur when defibrinated dogs were used he attributed it to intravenous clotting.

Levy (3) working with cats, found that varying percentages of chloroform produced different results. In point he states that the mammalian heart under the influence of chloroform is in an irritable condition the irritability being raised under conditions of light and lowered under deep anaesthesia. He also found that intravenous injection of adrenalin chloride under high percentages of chloroform produces a condition of irritability in the ventricle similar to that observed under low percentages of chloroform alone.

In the previous work with adrenalin I encountered this trouble whenever chloroform was used for anaesthesia. Upon post-mortem examination the heart was found dilated and occasionally fibrillating. These experiments were done in order to determine if possible the cause of death under chloroform following the injection of adrenalin.

The animals (dogs) were prepared in a manner similar to that described in a previous paper (4). In some experiments a small thistle tube connected to a recording tambour was inverted into the pericardial cavity and fixed there by a purse string suture in the pericardium. By means of this apparatus changes in the volume of the heart were easily recorded. As in previous experiments I used the tablet adrenalin of Parke, Davis and Company. Solutions varying in dilution from 1:10,000 to 1:100,000 were prepared and used in varying amounts. The experiments recorded here are typical of a series of twenty dogs.

Experiment I. A male dog weighing about 6 kgm. was used. All the figures given in this and the following experiments in regard to the blood pressure are in millimeters of Hg. The normal pressure was 120 systolic and 114 diastolic and the pulse 180. Stimulation of the right vagus with a very weak current totally inhibited respiration, but was without effect on the heart.

One cubic centimeter of 1:100,000 adrenalin was injected intravenously, the pressure rising to 142 systolic and 136 diastolic. The pulse accelerated slightly becoming 172. Respiration was not affected. After injecting 1 cc. of 1:10,000 adrenalin the pressure rose to 140 systolic and 130 diastolic and then suddenly dropped to 18 systolic with almost total inhibition of the heart. This was the most marked inhibition under ether anaesthesia I have seen produced by adrenalin. The heart recovered and in one minute the pressure had returned to 146 systolic and 140 diastolic, and then gradually declined to normal. Respiration was markedly inhibited.

One cubic centimeter of 1:100,000 adrenalin increased the pressure to 140 systolic and 126 diastolic. The pulse was 146 before and after the injection. Respiration was 46 and regular. One cubic centimeter of 1:10,000 adrenalin raised the pressure to 146 systolic and 152 diastolic. It then dropped to 90 systolic and 56 diastolic. Respiration was unaffected. A second injection produced like results.

The pressure was now raised artificially by clamping the abdominal aorta close under the diaphragm and above the coeliac axis to 140 systolic and 134 diastolic without any indication of slowing of the heart. Respiration, however, became augmented. Repetition of this procedure produced identical results.

Chloroform was now used instead of ether for anaesthesia.

One cubic centimeter of 1:100,000 adrenalin increased the pressure from 106 to 120 systolic without any evidence of change in the respiration on the heart rate. One cubic centimeter of 1:10,000 adrenalin caused a rise in pressure from 110 to 120 systolic. The heart slowed from 146 to 70 and the pressure dropped to 88 systolic and 60 diastolic. Following this, stimulation of the right vagus produced inhibition of

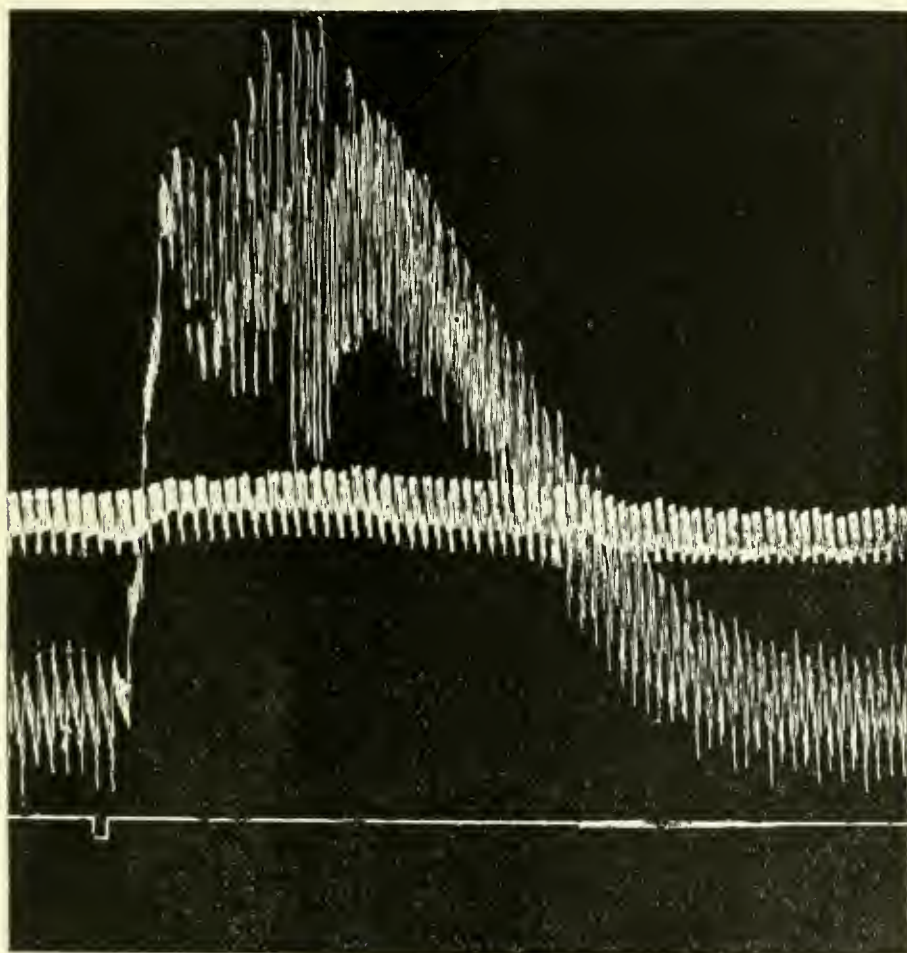


FIG. 1. RISE IN PRESSURE AND CHANGE IN VOLUME PRODUCED BY 1 CC. 1:10,000 ADRENALIN. ETHER ANAESTHESIA

the respiration and the heart slowed from 156 to 60. Both vagi were now cut. Injection of 1:10,000 adrenalin increased the pressure from 104 to 114 systolic. It then dropped to 80 systolic with marked inhibition of the heart rate changing from 136 to 62. Respiration was unaffected. Further injection of adrenalin was found to weaken the heart which post-mortem was found greatly dilated.

Experiment II. A male dog weighing 8 kgm. was used. The normal pulse was 110, systolic pressure 126. diastolic 112. Following the injection of 1 cc. of 1:10,000 adrenalin the heart rate accelerated to 210, the pressure rising to 230 systolic and 196 diastolic. There was

TABLE 1

PULSE	PRESSURE		REMARKS
	Systolic	Diastolic	
180	120	114	Normal
172	142	136	After 1 cc. 1:100,000 adrenalin
180	140	130	After 1 cc. 1:10,000 adrenalin
Inhibition	18		10 seconds afterwards
164	140	126	After 1 cc. 1:100,000 adrenalin
114	120		Stimulation of right vagus
			Chloroform used for anaesthesia
160	120	102	After 1 cc. 1:100,000 adrenalin
47	120	116	After 1 cc. 1:10,000 adrenalin
156			Before stimulation of left vagus
60			After stimulation of right vagus
			Vagi cut
136	104	98	Before 1 cc. 1:10,000 adrenalin
62	80	74	After 1 cc. 1:10,000 adrenalin

TABLE 2

PULSE	PRESSURE		REMARKS
	Systolic	Diastolic	
110	126	112	Normal
210	230	196	After 1 cc. 1:10,000 adrenalin
126	116	110	Before 2 cc. 1:10,000 adrenalin
162	240	200	After 2 cc. 1:10,000 adrenalin (chloroform)
100	138	130	Before 1 cc. 1:10,000 adrenalin
120	208	190	After 1 cc. 1:10,000 adrenalin. Further injections of adrenalin produced fibrillation

no evidence of slowing. After a second injection of 2 cc. of 1:10,000 adrenalin the rate increased from 126 to 162, systolic pressure from 116 to 240 and diastolic from 110 to 200. The heart volume was unaffected and the respiration became shallower.

Chloroform was now used for anaesthesia. The volume of the heart almost immediately began to increase, indicating dilatation due to the chloroform. An injection of 1 cc. of 1:10,000 adrenalin increased the pressure from 138 to 208 systolic, the rate changing from 100 to 120. With the rise the volume became less. However the pressure began to fall and synchronously the volume increased and the heart

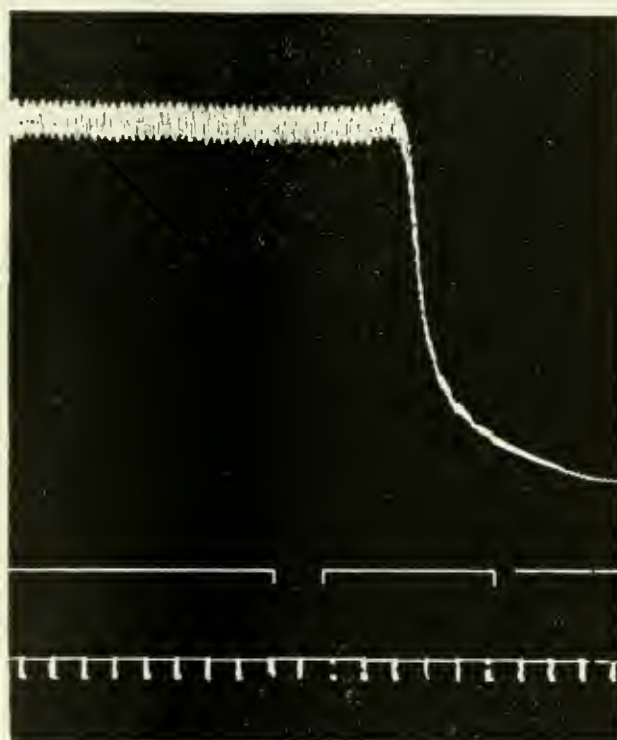


FIG. 2. EFFECT OF 0.1 CC. CHLOROFORM INJECTED INTRAVENOUSLY

TABLE 3

PULSE	PRESSURE		REMARKS
	Systolic	Diastolic	
150	94	86	Normal
Imperceptible	12		After one minim of chloroform
120	40	30	After massage
90	74	56	After 1 cc. 1:10,000 adrenalin
Imperceptible	18	16	100 seconds later

action became weaker. An injection of 1 cc. of 1:10,000 adrenalin was given but the tendency to dilatation remained. The pressure again suddenly fell and the heart dilated. Further injection of adrenalin tended to weaken the heart, the period of primary stimulation becoming shorter. The heart was found enormously dilated and apparently fibrillating.

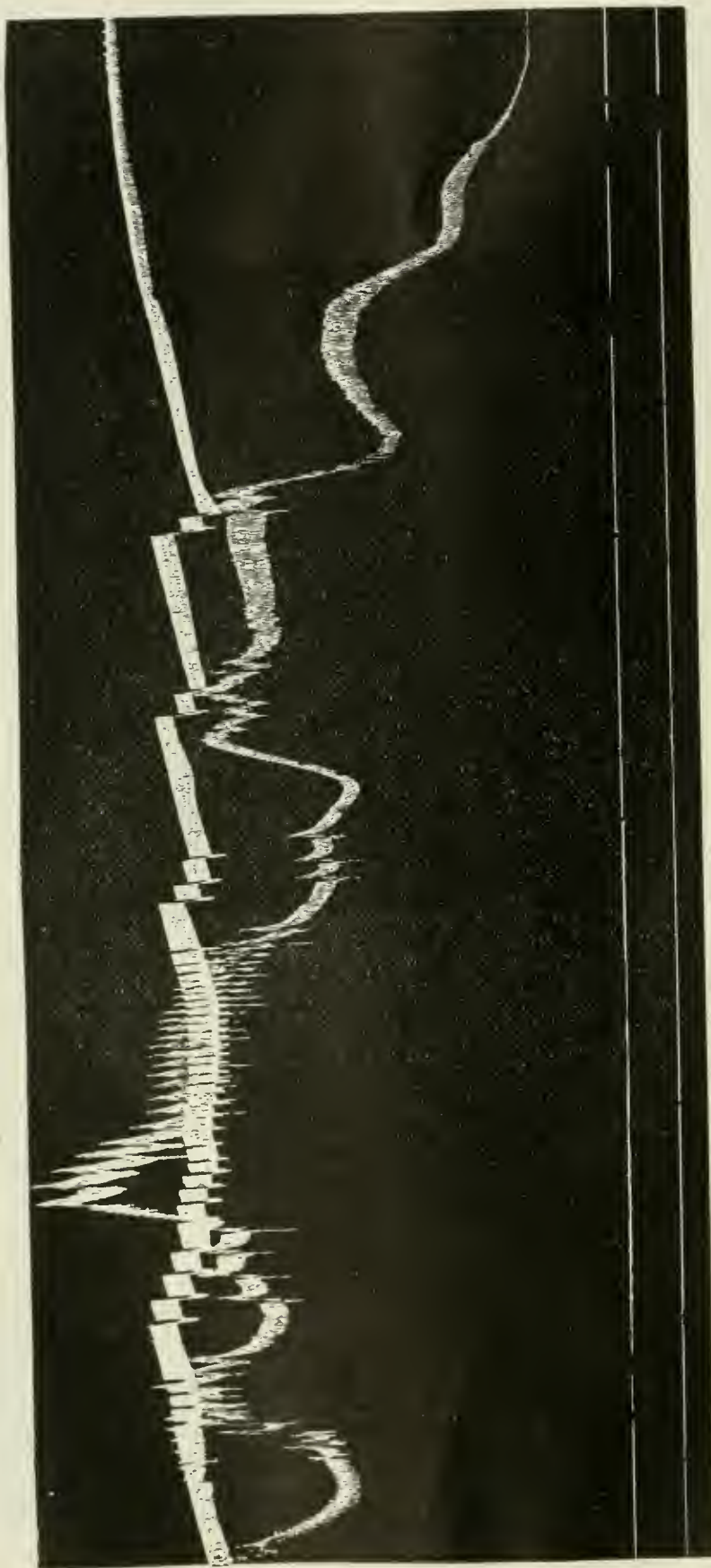


FIG. 3. CHANGES IN PRESSURE AND HEART VOLUME PRODUCED BY CHLOROFORM AND ADRENALIN

Experiment III. A female dog weighing 2 kgm. was used. The normal pulse was 150, systolic pressure 94 and diastolic 86. One-tenth cubic centimeter of chloroform was injected intravenously. Following a slight rise, the pressure abruptly fell to 12 systolic and the heart beat became almost imperceptible. Injection of adrenalin as the pressure fell was without effect. This was due in all probability to the fact that the drug did not reach the heart because of the weak pulse and the low pressure. After vigorous massage the heart rate increased

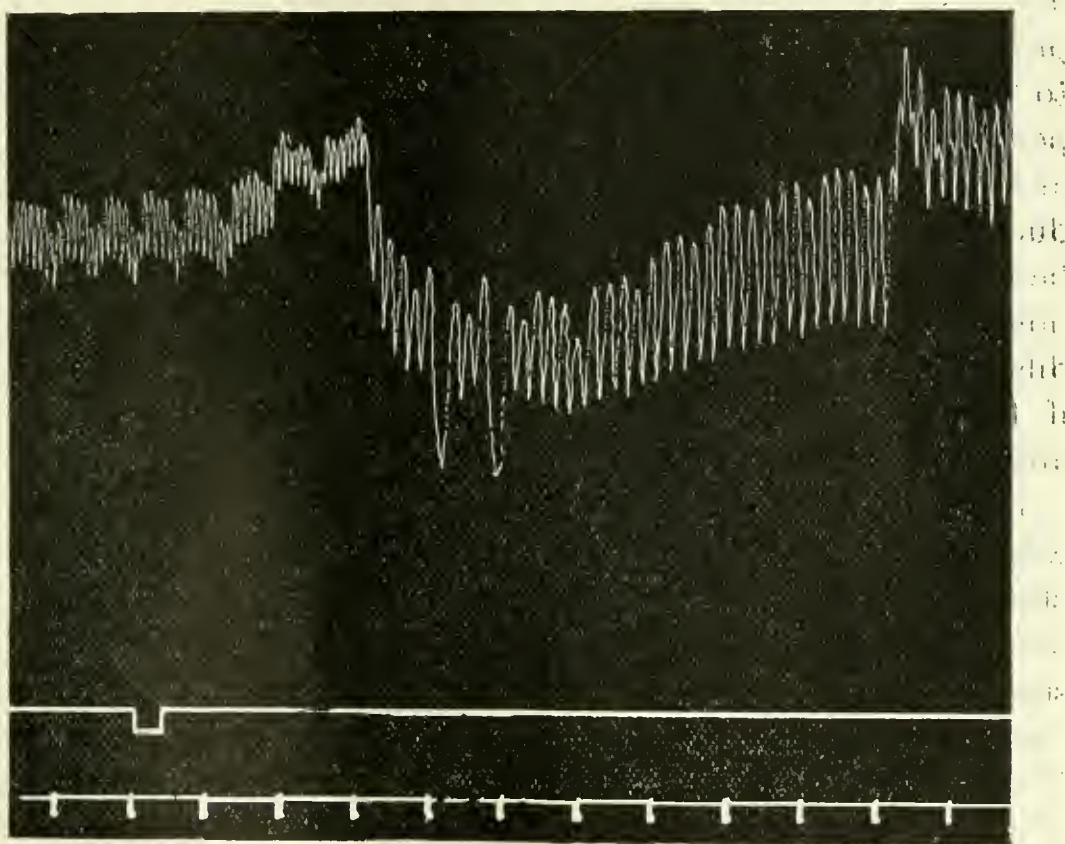


FIG. 4. INHIBITION PRODUCED BY 1 CC. 1:10,000 ADRENALIN AFTER SECTION OF VAGI

though the pressure tended to remain low. Anaesthesia was discontinued here and not used throughout the remainder of the experiment. The pulse was 120, the systolic pressure 120 and the diastolic 30. One cubic centimeter of 1:10,000 adrenalin was injected. The pressure rose and very slight inhibition was effected. It then gradually fell and the heart became weaker and practically stopped. Cardiac massage again effected a strengthened and increased beat, but small doses of adrenalin regularly caused the heart to stop. Section of the vagi was without avail indicating that the action is peripheral.

DISCUSSION

These experiments show that chloroform when inhaled or injected intravenously produces a toxic effect on the heart resulting in dilatation and permanent weakness. This action is direct since it occurs after section of the vagi and after atropine. The heart is slowed because of this toxic condition.

In a few experiments in which I have injected small amounts 0.1 cc. of chloroform intravenously I have found that the blood pressure after a very short and slight rise immediately began to fall and the heart to weaken. If adrenalin be injected at this point the heightened blood pressure induces paralytic dilatation and occasionally fibrillation. In one experiment atropine produced identical results. In all the cases examination of the heart post-mortem showed a dilated organ. Resuscitation cannot be effected when once the heart has become paralytically dilated and ventricular fibrillation has supervened. However, if fibrillation has not occurred cardiac massage may restore automatic beating.

Levy (3) the only investigator that I have been able to find who has determined any definite relation between adrenalin and chloroform states that ventricular fibrillation is produced by cardiac stimulation under light chloroform anaesthesia, and that small doses of adrenalin chloride under high percentages of chloroform vapor produce a condition of irritability in the ventricle similar to that observed to result under low percentages of chloroform alone. He also states that low tensions of chloroform administered to cats together with adrenalin chloride produces fibrillation. He attributes death to the low chloroform which occurs at the beginning and the end of administration and which produces an irritable heart which tend to fibrillate. Sensory stimulation either reflexly from the struggling or from the action of the vapor on the respiratory passages produces fibrillation.

In view of these facts Hare (6) states that chloroform as an anesthetic is contraindicated after adrenalin has been used hypodermically or locally and that chloroform if used in such a case should be preceded by atropin to nullify the action of the adrenalin.

Elliott noted that dogs under ether despite section of the vagi died suddenly due to heart failure with sudden injection of adrenalin. He found large clots in the innominate vein and the right heart and since death did not occur when defibrinated dogs were used he attributed it to the intravenous clotting.

Permanent inhibition of the heart under chloroform anaesthesia and following the injection of adrenalin, I think is due primarily to the weakening and the dilatation induced by the chloroform. While stoppage of the heart occurs more quickly and easily under light anaesthesia than under deep anaesthesia I have found complete inhibition to result under the latter condition. Adrenalin to a small degree causes distention of the heart.

Adrenalin by increasing the peripheral pressure causes an extremely high aortic pressure against which the heart cannot empty itself. When the heart muscle is weakened as by chloroform a probable dilation is obvious. Adrenalin at the same time by stimulating the sympathetic endings and the heart muscle directly tends to augment and accelerate the heart beat, but in consequence of the high aortic pressure and the toxic effect of the chloroform on the heart which causes it to dilate, the muscle fibers losing their normal elasticity, this action of adrenalin throws an added load on the already weakened heart and induces dilatation and sometimes fibrillation.

In a series of experiments I injected aconite with hope of producing fibrillation and of determining whether dilatation preceded or followed fibrillation. After adrenalin the heart would weaken and stop, dilating before fibrillation occurred. I believe that paralytic dilatation occurs first and that ventricular fibrillation which may supervene on this condition is due to active centers in the heart attempting to reestablish a sequence of beats. I do not believe this to be a compensatory dilation, the heart dilating so that at each beat a greater amount of blood is forced into the system. I am of the opinion that this dilation is toxic or paralytic in character and due to chloroform acting directly on the muscle. It does not occur when the pressure is lowered by other means. It is proportional to the amount and the length of time that the chloroform is administered. It is terminal in character and recovery from it is almost impossible.

Although Gottlieb (6) working with dogs found that adrenalin injected intravenously would resuscitate a heart stopped by chloral hydrate and on this basis suggested that it might be used in chloroform narcosis with threatened heart collapse. I think that adrenalin is contraindicated wherever chloroform is used and that chloroform, wherever adrenalin has been employed. That chloroform weakens the heart muscle permanently is proven by experiment II. Cardiac massage effected resuscitation because it tended to contract the heart and relieve the distention caused secondarily by the blood collecting in the already dilated and weakened heart. As soon as adrenalin was injected the extra load caused the heart to again dilate.

That the blood pressure alone bears no definite reflex relation to the degree of slowing I think is amply proven by my experiments. In experiment I the pressure was raised artificially to a height equal to that produced by 1 cc. of 1/10,000 adrenalin without any evidence of slowing although the adrenalin effected inhibition. One cubic centimeter of 1/100,000 adrenalin raised the pressure to a similar height without an inhibition indicating that the size of the dose is a determining factor. That the increased pressure stimulating the inhibitory center and hence causing slowing is not the causative factor is proven by the fact that the phenomenon may occur after section of the vagi. I have found repeatedly the inhibition occurring after the pressure had returned to normal. In some cases the pressure was increased to 300 mm. Hg. without any slowing being evident.

It may be stated, however that the higher the pressure the more easily does the action occur. I think it is the direct action, i.e., the high aortic pressure preventing the heart from emptying itself that is paramount in producing these changes; and while the heart weakened by chloroform dilates more readily, the weakness would soon be adjusted if the great injury produced by the high pressure was not added.

CONCLUSIONS

1. Chloroform is toxic for heart muscle, producing or tending to produce weakening of the organ.

2. Inhibition under chloroform anaesthesia after adrenalin is due primarily to the toxic or paralytic dilatation of the heart, ventricular fibrillation supervening on this condition.

3. Because of the action of chloroform on the heart adrenalin is contraindicated wherever chloroform is employed and chloroform wherever adrenalin is used.

4. The blood pressure has no definite reflex relation to the production of the condition of paralytic dilatation but has a most important direct action by preventing the ventricle from emptying itself.

5. The adrenalin action is peripheral since it occurs after section of the vagi.

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THE TOXICITY AND SKIN IRRITANT EFFECT OF CERTAIN DERIVATIVES OF DICHLOROETHYL SULFIDE

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In view of the interesting physiological effects of dichloroethyl sulfide as brought out by various studies during the recent war, considerable interest attaches itself to the pharmacological and toxicological properties of derivatives of this substance. An opportunity to examine certain of its derivatives has been afforded us through the kindness of Prof. E. E. Reid and Dr. O. B. Helfrich of the Chemical Department of the Johns Hopkins University, who were interested in the chemistry of this substance. We wish here to thank them for furnishing samples of the compounds used in this investigation. The methods of preparation and properties of the various substances will be found in a recent article by Helfrich and Reid (1).

Dichloroethyl sulfide is a very poisonous substance and exerts a local action on the respiratory tract, eyes, and skin, and a general systemic action when absorbed or injected into the circulation in sufficient quantity (2). Its effects upon the skin consist of hyperemia, edema, vesication, and necrosis leading to a skin lesion of great chronicity (3). Only one or more of the effects named may be present depending on the amount or concentration of the substance used. The theory of an intracellular liberation of hydrochloric acid has been advanced to explain the action of dichloroethyl sulfide (2). This has received support from the work of Lillie, Clowes, and Chambers (4) on marine

¹ A few preliminary experiments were carried out by the senior author in the Pharmacological Laboratory of the Johns Hopkins University.

eggs. It was thought that aside from the interesting comparison of the action of these closely related compounds, some data might be accumulated for further testing this theory.

The present investigation has comprised mainly an examination of the toxicity of the compounds for white mice, their effects on the human skin, and their antiseptic action. The great power of penetration possessed by *mustard gas* suggested that some of its derivatives containing antiseptic groups (e.g., phenols, naphthols, etc.) might prove valuable as antiseptics.

A number of the substances furnished us by Reid and Helfrich proved so slightly soluble in water, olive oil or alcohol that they had to be discarded. A few experiments were carried out with them upon bacteria and unicellular organisms, but showed nothing conclusive or of interest, so are not included in this report. It is rather unfortunate that these compounds could not be included as they make a very complete series of derivatives as shown in the summary in the article of Helfrich and Reid. The slight solubility in water and also lack of sufficient material prevented us from making our experiments more extensive.

The derivatives of dichloroethyl sulfide included in the present study were of two main types, those formed by reactions involving the chlorine atoms, substituting various groups in their place; and those involving the sulphur atom, raising it to a higher state of oxidation (1).

TOXICITY FOR MICE

The compounds were dissolved in olive oil, usually in 1 or 2 per cent concentration, and injected in varying amounts subcutaneously into white mice. Six to twelve mice were used for each compound. The animals were kept under observation for six or seven days, but practically all deaths occurred within forty-eight hours after injection. Control injections of 1 cc. of olive oil had no effect on the mice. This was more than was ever used in injecting the compounds. The following table summarizes the results obtained. The minimum lethal dose is given in milligram per kilo of mouse. Dichloroethyl sulfide is

included for comparison. In certain cases the minimum lethal dose was not determined because of the limited solubility of the compound in olive oil; this would have necessitated the injection of too large an amount of fluid.

TABLE 1
Toxicity for mice

NUMBER	NAME	FORMULA	MINIMUM LETHAL DOSE PER KILOGRAM
			<i>mgm.</i>
1	Dichloroethyl sulfide.....	$(\text{ClCH}_2\text{CH}_2)_2\text{S}$	125
2	Bis (β -chloro-ethyl) sulf- oxide.....	$(\text{ClCH}_2\text{CH}_2)_2\text{SO}$	125
3	Bis (β -chloro-ethyl) sulfone.	$(\text{ClCH}_2\text{CH}_2)_2\text{SO}_2$	105
4	Bis (β -iodo-ethyl) sulfoxide.	$(\text{ICH}_2\text{CH}_2)_2\text{SO}$	150
5	Bis (β -iodo-ethyl) sulfone...	$(\text{ICH}_2\text{CH}_2)_2\text{SO}_2$	30
6	Bis (β -thio-ethyl acetate)...	$(\text{CH}_3\text{COOCH}_2\text{CH}_2)_2\text{S}$	> 850
7	β -bromethylbutylsulfide*....	$\text{BrCH}_2\text{CH}_2\text{-S-CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	490
8	Bis (β -ethyl-mercapto-ethyl) sulfide.....	$(\text{EtSCH}_2\text{CH}_2)_2\text{S}$	> 650
9	Bis (β -ethyl-mercapto-ethyl) sulfone.....	$(\text{EtSCH}_2\text{CH}_2)_2\text{SO}_2$	175
10	Bis (β -propyl-mercapto- ethyl) sulfone.....	$(\text{PrSCH}_2\text{CH}_2)_2\text{SO}_2$	300
11	Bis (β -butyl-mercapto-ethyl) sulfone.....	$(\text{BuSCH}_2\text{CH}_2)_2\text{SO}_2$	400
12	Bis (β -isobutyl-mercapto- ethyl) sulfone.....	$(\text{i-BuSCH}_2\text{CH}_2)_2\text{SO}_2$	500
13	Bis (β -phenoxy-ethyl) sulfide	$(\text{PhOCH}_2\text{CH}_2)_2\text{S}$	> 550
14	Bis (β -phenoxy-ethyl) sul- fone.....	$(\text{PhOCH}_2\text{CH}_2)_2\text{SO}_2$	> 550
15	Bis (β -phenyl-mercapto- ethyl) sulfide.....	$(\text{PhSCH}_2\text{CH}_2)_2\text{S}$	> 600
16	4-phenyl-1,4-sulfanazan.....	$\text{PhN} < (\text{CH}_2\text{CH}_2)_2 > \text{SO}$	> 650
17	4-p-cresyl-1,4-sulfonazan....	$\text{p-CH}_3\text{C}_6\text{H}_4\text{N} < (\text{CH}_2\text{CH}_2)_2 > \text{SO}_2$	> 450

* This substance is not described in the article by Helfrich and Reid. It was kindly furnished us by Reid and Whitner, and will be described by them in an article soon to be published.

EFFECT ON THE SKIN

The effect of the various compounds listed in table 1, upon the human skin has been examined. Since it has been clearly demonstrated that great individual susceptibility of the skin

exists for dichloroethyl sulfide, and there is no reason to suppose that such does not exist for the compounds here studied, it would be impossible to determine quantitatively the relative potency of these substances as skin irritants without a very large number of observations. We were not in a position to make such observations but have contented ourselves with examining the effects upon the authors and in certain cases upon four or five other individuals.

The results of the study of the effect of these compounds on the skin were briefly as follows. Dichloroethyl sulfide and dichloroethyl sulfone (no. 3) are very active skin irritants and produce similar effects. The former appears to be more active than the latter. Diiodoethyl sulfone (no. 5) is probably in the same class, but its limited solubility prevented a complete comparison. The *diacetate* (no. 6) and bromethylbutyl sulfide (no. 7) are only very slightly irritating in comparison with *mustard gas*, but the pure liquids resemble very dilute concentrations of the latter in their effects. The *sulfoxides* of the chlorine and iodine derivatives (nos. 2 and 4) can scarcely be called irritating to the skin. The remainder of the compounds examined have not been found to be irritating in the concentrations used.

The substances were applied to the skin dissolved in water, alcohol or olive oil, and in the case of substances liquid at ordinary temperatures, the pure liquid was applied. The *chlorosulphoxide* and *sulphone* act very differently upon the skin, the marked irritant properties of the *sulfide* appear to be lost in the former, but regained in the latter. These were tried upon six individuals in five per cent solution in alcohol. In all cases the *sulfone* produced "burns" similar in appearance to those of *mustard gas*, and in three instances typical vesicles resulted. The *sulfoxide* produced no effect in five instances, but in one case a mild erythema resulted from its application (the same result was obtained in this individual with a one per cent alcoholic solution). Aqueous and olive oil solutions of the *sulfone* were also active in producing skin irritation. A 1 per cent solution in olive oil produced a positive reaction in only one out of four individuals tested. Since dichloroethyl sulfide produces a positive result in 1 per cent olive oil solution in about 60 to 70 per cent of white men, it appears that the *sulphone* is somewhat less active on the skin than *mustard gas* (5).

The *iodo-sulphoxide* and *iodo-sulphone* appears to resemble the corresponding chlorine compounds. The former does not appear to irritate the skin, while the latter produced a positive reaction in one-half per cent olive oil solution in one out of three individuals tried. The comparative insolubility of the *iodo-sulphone* prevented further work with this substance.

Bis (β -thioethylacetate) in all cases tried produced only an erythema when applied to the skin as the pure liquid. A concentrated alcoholic solution spilled accidentally caused a marked irritation of the fingers with papular eruptions but without definite vesication. It was very similar, however, to finger "burns" previously experienced with very small amounts of *mustard gas*. This compound although slightly irritating to the skin is not comparable to *mustard gas* or the *sulphone*.

The bromethylbutyl sulfide applied to the skin as a pure oil produced only erythema with a slight papular eruption, but no vesication. A 2 per cent solution in olive oil produced no effect on the skins of two individuals.

The remainder of the compounds listed in table I were applied to the skin as two per cent solutions in olive oil with the exception of nos. 9 and 17 which were used in 1 per cent solutions. These were practically saturated solutions of the compounds in olive oil. No effects were noticed from any of the solutions.

ANTISEPTIC ACTION

All of the substances mentioned in table 1 with the exception of nos. 1 and 7 were tested for antiseptic and bactericidal activity against *Bacillus coli* and *Staphylococcus aureus*. These determinations were made by Miss Marian M. Crane of this laboratory and we wish to thank her for the summary of the results reported.

Saturated aqueous solutions of the compounds (usually with an excess of the undissolved substance) were inoculated from a twenty-four hour broth culture of *B. coli* and *Staphylococcus aureus*. After one hour and after twenty-four hours transplants were made on agar. Practically no antiseptic action was apparent in any of the compounds with the exception of dichloro-ethyl sulfone and diiodoethyl sulfone. A comparison of these with the corresponding *sulphoxides* was made. *The sulphones are many times more toxic for bacteria than the sulfoxides.*

To 1 cc. of the solution of the compound was added one loop of a twenty-four hour broth culture of *B. coli*. Transplants on agar were made after twenty-four hours. A saturated solution of the *chloro-sulfoxide* (about 1 per cent) showed growth but not as great as the control. No growth was obtained from a 1:32 dilution of a saturated solution (about 0.5 per cent) of the *chloro-sulfone* and only three colonies from a 1:64 dilution. Therefore, the *sulfoxide* required a greater concentration than 1:100 to kill, while the *sulfone* killed in about 1:10,000. The *sulfone* is about 100 times as toxic as the *sulfoxide* for bacteria.

To 2 cc. of the solution were added two drops of a twenty-four hour broth culture of *Staphylococcus aureus*. Transplants were made on agar after twenty-four hours. A saturated solution of the *iodo-sulfoxide* permitted growth, while a saturated solution of the *iodo-sulfone* killed the organisms and from a one-half saturated solution the transplant gave only three colonies, the control giving more than could be counted. The solubilities of these two compounds were not determined, but the *sulfone* is practically insoluble in water and the *sulfoxide* moderately soluble (1). The much greater toxicity of the *sulfone* for bacteria is thus apparent.

ACTION ON PARAMECIUM

In order to study the action of these compounds as protoplasmic poisons in distinction from any action they might exhibit as selective poisons when injected into an animal, an attempt was made to use a unicellular organism—*Paramecium caudatum*. On account of the very slight solubility of most of the derivatives in water, it was impossible to compare the toxicity of the whole series on these organisms. However, the results obtained with the *chloro-sulfoxide* and *chloro-sulfone* (which are the most soluble of the compounds in water) are interesting. The data indicate very clearly that the *sulfone* is very much more toxic for these organisms than is the *sulfoxide*. Figures on the toxicity of dihydroxyethyl sulfide, one of the hydrolytic products of *mustard gas* which has previously been shown to be non-toxic for dogs (6) and on dichloroethyl sulfide are included for comparison. It is obvious from the results that the *sulfide* and *sulfone* are of the same order of toxicity as protoplasmic poisons, while the *sulfoxide* and *hydroxy-sulfide* are relatively non-toxic. Lillie, Clowes, and Chambers in their study of the action of *mustard*

gas on marine organisms found it highly toxic, while the *hydroxy-sulfide* was practically non-toxic (4).

To 2 cc. of the solution of the substance to be tested was added 0.1 cc. of a thick suspension of *paramecia* obtained by centrifuging, and the organisms observed with a hand lens. Varying dilutions of the different substances were used. The results reported are the averages of a number of experiments with the *sulfoxide* and *sulfone*, and of two each with the *sulfide* and *hydroxy-sulfide*. Comparative experiments were always made at the same time to avoid any differences in the susceptibility of the organisms. The figures for the *sulfide* cannot be accurately determined on account of its rapid hydrolysis in water, and also because a dilute buffer mixture of phosphates was necessary in working with this substance to avoid the effect of the extra cellular acid liberated from it by hydrolysis. The presence of phosphate or in fact of alkali increases the toxicity of all the substances slightly, but control experiments with the *sulfoxide* and *sulfone* showed that the error from this source was not appreciable.

The *sulfone* killed in a concentration of 1:600 in one hour, and 1:20,000 to 1:80,000 in twenty-four hours; the *sulfoxide*, in a concentration of 1:100 in one hour, and 1:125 in twenty-four hours; the *hydroxy-sulfide*, in a concentration of 1:300 in one hour, and 1:600 in twenty-four hours.

Using a dilute phosphate mixture as buffer, the *sulfone* killed in a concentration of 1:20,000 in one hour, and 1:150,000 in twenty-four hours; the *sulfide*, in a concentration of 1:40,000 in one hour and 1:80,000 in twenty-four hours. In control experiments, the organisms lived in the phosphate mixture longer than forty-eight hours.

SOLUBILITY AND HYDROLYSIS

In view of the theory which has been proposed to explain the action of dichloroethyl sulfide, which correlates its action with its lipoid solubility and ease of hydrolysis into hydrochloric acid, it appeared interesting to obtain certain data on the hydrolysis and solubility of compounds which can theoretically yield an acid on hydrolysis (the first seven in table 1). Helfrich and Reid (1) give in their article certain data on these questions. They find the *chloro-sulfoxide* to be much more soluble in water than the *chloro-sulfone*, and somewhat less soluble in alcohol. They also have determined that the *sulfone* is much more readily

hydrolized than the *sulfoxide*. The bis- (β -thio-ethyl acetate) can be quantitatively hydrolized by alkali.

The hydrolysis of dichloroethyl sulfide has been carefully studied by Hopkins (7). It is hydrolized to the extent of about 50 per cent in ten minutes at 20°, 95 per cent at 37.5° and only 5 per cent at 0°. The dichloroethyl sulfone is slowly hydrolized in aqueous solution at 20°, while the *sulfoxide* is not, as shown by conductivity measurements. A solution of the *sulfone* heated to boiling gives a distinct precipitate with silver nitrate, while a boiling solution of the *sulfoxide* does not (1). The *sulfone* is very readily and completely hydrolized at room temperature by weak alkali. Under conditions of alkalinity as present in the animal organism, the *sulfone* is hydrolized, while the *sulfoxide* is not.

Twenty cubic centimeters of an aqueous solution of the *sulfone*, containing 1 mgm. per cubic centimeter were mixed with 10 cc. of a phosphate mixture (pH = 7.5) and the hydrogen ion concentration determined colorimetrically at various intervals of time. In one hour the value of pH had become 7.0 and eighteen hours later it was 6.6. At this time a determination of the chloride content of the solution indicated complete hydrolysis of the *sulfone*. A similar experiment was carried out under the same conditions with the *sulfoxide*. During 18 hours, the pH remained constant at 7.5, and at end of the experiment no precipitate could be detected in the solution on the addition of nitric acid and silver nitrate.

The following experiment indicates roughly the rate of hydrolysis of the *sulfone* in a hydrogen ion concentration corresponding to that of the blood. To 200 cc. of a phosphate mixture (pH = 7.7 and $\frac{M}{30}$) were added 100 cc. of a solution of the *sulfone*. The chloride was determined by the Volhard method in 25 cc. portions (1 cc. AgNO_3 = 8.23 mgm. sulfone). Temperature, 22°.

TIME	AgNO ₃ REQUIRED	pH	HYDROLIZED
	cc.		per cent
1 min.	0.10	7.70	5.9
8 min.	0.15	7.70	8.8
30 min.	0.40	7.60	23.5
63 min.	0.60	7.55	35.8
150 min.	0.95	7.45	55.9
18 hrs.	1.70	7.30	100.0

The *diiodoethyl sulfone* gives a precipitate when heated with trace of alkali and treated with nitric acid and silver nitrate, while under the same conditions the *sulfoxide* does not.

The *bis-thio-ethyl acetate* is quantitatively hydrolized when heated with alkali (1). In aqueous solution, it appears to be hydrolized until equilibrium is established when about 15 per cent of the ester has been decomposed.

To 1000 cc. of water were added about 700 mgm. of the *di-acetate*. Complete solution occurred on shaking. Portions of 100 cc. were withdrawn at intervals, and titrated with 0.0112 N alkali and phenolphthalein. Temperature, 21° to 23°.

TIME	KOH REQUIRED	HYDROLIZED
	cc.	per cent
2.5 min.	5.0	8.0
12.5 min.	6.4	10.1
42.0 min.	7.4	11.7
147.0 min.	7.5	11.9
295.0 min.	7.6	12.0
24 hrs.	8.7	13.8

One hundred cubic centimeters completely hydrolized by heating with alkali required 63.0 cc. 0.0112 N potassium hydroxide.

Bromethylbutyl sulfide in aqueous solution is hydrolized extremely rapidly even more so than *mustard gas*—over 95 per cent in ten minutes at 20°. It is only very slightly soluble in water, and when an excess is added to water it is only slowly hydrolized resembling *mustard gas* in this respect.

To 2 litres of water was added about 300 mgm. of bromethylbutyl sulfide, and the mixture shaken vigorously. A small amount of the oil remained undissolved. A sample of 100 cc. withdrawn six minutes after mixing required 4.2 cc. of 0.0112 N potassium hydroxide. A sample nineteen minutes later required 4.4 cc. This appeared to represent complete hydrolysis as judged by subsequent titrations.

Two hundred milligrams of the oil were mixed with 250 cc. of water in a bottle. A large amount of the material remained undissolved. The mixture was allowed to stand for several days with occasional

shaking. The undissolved oil gradually disappeared. Seven days after mixing, a portion of the mixture was analyzed for hydrobromic acid by the Volhard method. The amount of hydrobromic acid formed corresponded to 194.7 mgm. of the original substance, indicating complete hydrolysis.

Dichloroethyl sulfide is soluble in water to the extent of 0.07 per cent at 10° (7). The *chloro-sulfoxide* is soluble to 1.2 per cent; and the *sulfone*, 0.6 per cent at 20° (1). The *iodo-sulfoxide* is moderately soluble in hot water, while the *iodo-sulfone* is practically insoluble (1). The *di-acetate* is soluble to a somewhat greater degree than 0.15 per cent at room temperature, while *bromethylbutyl sulfide* is soluble at room temperature to the extent of somewhat less than 0.01 per cent.

The partition coefficients of some of the compounds were determined using xylene and water at room temperature. The *sulfoxide* and *sulfone* are fairly stable in neutral solution and this value can be easily determined, while the *di-acetate* and *monobrom* compounds are hydrolyzed so rapidly that the determination of this value is besought with the same difficulties as in the case of *mustard gas*. The concentrations of the various substances for which the values of the partition coefficients were determined varied between 0.3 and 0.5 per cent. The following values were found for the partition coefficients: *mustard gas* greater than 200 (5); *chloro-sulfoxide*, 0.5; *chloro-sulfone*, 4.6; *di-acetate*, 10; *bromethylbutyl sulfide*, greater than 1500.

DISCUSSION

An examination of the toxicity for mice of the compounds listed in table 1, brings out some interesting comparisons. In general the compounds containing halogen are the most toxic, and those containing aliphatic radicals in place of the chlorine are more toxic than those containing aromatic nuclei. Bromethylbutyl sulfide is less toxic, however, than several of the substances not containing halogens. The sulphones are more toxic than the corresponding sulfides. Increase in molecular weight decreases the toxicity (see nos. 9, 10, 11). The *iodo-*

sulfone is about three times as toxic as the corresponding chlorine compound. The toxicity of *mustard gas* (no. 1) is not as great in this series as would have been expected. It is scarcely more toxic than the *chloro-sulfoxide* and *sulfone*, and the *iodo-sulphoxide* while it is only about a fourth as toxic as the *iodo-sulfone*. *Mustard gas* is fatal for dogs on intravenous injection in a dosage of 6 mgm. per kilo, while here it takes 125 mgm. per kilo to kill mice on subcutaneous injection.

The method of subcutaneous injection of oil solutions is not at all satisfactory for comparing the toxicity of these compounds. Differences in absorption and the possible hydrolysis of certain of the compounds before they are absorbed may account for some of the differences noted. Intravenous injection of aqueous solutions would be the most exact method of comparison. This was impossible in the case of most of the substances, on account of their slight solubility in water. The minimum lethal dose of the *chloro-sulfoxide* on intravenous injection into mice was found to be 120 mgm. per kilo, and of the *chloro-sulfone* 80 mgm. per kilo. These figures are not far different from those obtained on subcutaneous injection.

In determining the toxicity of substances for a complicated organism as a mammal, a great many factors may contribute to this effect. Two poisons may be equally toxic, but have entirely different effects, e.g., one acting on the heart primarily and another on the central nervous system. A poison may be very toxic for a certain specialized form of mammalian protoplasm, and yet comparatively non-poisonous for protoplasm in general. In other words, the question of selective poisons and general protoplasmic poisons must be considered. This is well illustrated by a consideration of the action of the *chloro-sulfoxide* and *sulfone*. Both are of the same general order of toxicity for mice, but the latter is a violent skin irritant while the former scarcely irritates at all, and the latter is over a hundred times as toxic as the *sulfoxide* for uni-cellular organisms, bacteria and paramecia. In attempting to correlate the action of this series of compounds with their ease of hydrolysis into an acid, and their solubility in water and lipoids, the fairer method would appear

to be to consider their effects on the skin and *general protoplasmic* toxicity rather than their subcutaneous toxicity for mice.

Dichloroethyl sulfone hydrolyzes somewhat slower than *mustard gas*, but has a considerably lower partition coefficient. This latter would somewhat compensate for its slower hydrolysis as it would be given up from lipoids into the aqueous phase more rapidly. It acts similar to *mustard gas* on the skin, and is of the same order of toxicity for protoplasm. The *chloro-sulfoxide* does not appear to hydrolyze and neither irritates the skin nor is toxic for protoplasm. It is only slightly less toxic than the *sulfone* for mice and one would expect the two compounds to be entirely different in their mechanism of producing death. Since a larger supply of these two compounds has been obtained, a careful study of the pharmacological action of the two will be made. As far as they have been studied the *iodo-sulfoxide* and *sulfone* resemble the corresponding chlorine compounds. Bis-thio-ethyl acetate and bromethylbutyl sulfide both hydrolyze rapidly in aqueous solution giving rise to acetic and hydrobromic acids respectively. Neither are very active as skin irritants, nor very toxic for mice. One might interpret these facts at first glance as in utter disagreement with the theory of intracellular acid production as explaining the action of *mustard gas*. However, when it is considered that a certain threshold of acid has to be reached in the cell before any effects are apparent (8), an explanation is readily offered. The *di-acetate* gives rise to acetic acid, which is known to be converted by the organism almost completely into carbonate. Acetic acid in the cell would presumably be neutralized by the reserve alkali and then changed to carbonate, if the entrance of acid was not too rapid. The hydrogen-ion concentration of the cell would not be appreciably affected. The threshold for the *acetate* would be much higher than for compounds which give rise to an inorganic acid, which cannot be changed chemically by the cell, but is neutralized and excreted. This threshold for the skin in the case of *mustard gas* vapor has been shown to be about 0.002 to 0.005 mgm. per litre of air (8). Bromethylbutyl sulfide is hydrolyzed much more rapidly than *mustard gas* but is comparatively inactive on the

skin when compared with the latter. Its solubility in water is very much less than dichloroethyl sulfide, however, while its solubility in lipoids is greater. Its partition coefficient is extremely high, much higher than that of *mustard gas*, although neither can be determined with any high degree of accuracy due to the rapid hydrolysis in the aqueous phase. In a previous paper, a rather complete discussion was presented on the mechanism of absorption of mustard gas by the skin⁽⁸⁾. This discussion involved a consideration of a three phase system, the air over the skin surface, the outer phase; a central lipoidal phase; and an inner aqueous phase. The great differences observed in the sensitivity of the skin of different individuals (5) was explained on this basis. The high partition coefficient of bromethylbutyl sulfide would retard its delivery into the aqueous phase, where it would be readily hydrolyzed, and thus prevent the threshold concentration of acid being reached within the cell. When we also consider that less than one-half as much acid is produced from a given weight of this compound as from *mustard gas*, it is not surprising that the pure liquid is only mildly irritating to the skin. The volatility of the compounds is another property which should be taken into account in attempting to correlate their action with their ease of hydrolysis and lipoid and water solubility. Since no determinations of vapor pressure are available we cannot discuss this point.

SUMMARY

1. The toxicity on subcutaneous injection into mice has been determined for a number of derivatives of dichloroethyl sulfide. Certain relations between toxicity and chemical constitution have been pointed out.

2. The skin irritant effect of these compounds has been examined on man. Dichloroethyl sulfone is of the same order of activity as dichloroethyl sulfide, while dichloroethyl sulfoxide is practically inert. The former hydrolyzes readily in weak alkali, while the latter does not. The activity of the other compounds has been discussed.

3. The compounds have been examined for their antiseptic properties.

4. The toxicity of dichloroethyl sulfoxide and dichloroethyl sulfone, while of the same magnitude for mice, is markedly different for unicellular organisms, the latter being a hundred times more toxic than the former.

5. The solubility of certain of the derivatives in water and xylene, as well as their rapidity of hydrolysis to yield an acid has been determined. An attempt has been made to correlate with these properties their physiological action as far as determined.

6. The evidence obtained as far as it goes is not in disagreement with the theory of intracellular acid production previously proposed to explain the action of *mustard gas*. Considerable support is given the theory by the study of the dichloroethyl sulfoxide and sulfone.

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STUDIES OF CHRONIC INTOXICATIONS ON ALBINO RATS

I. ORGANIZATION OF THE INVESTIGATIONS

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INTRODUCTION

Chronic intoxications have considerable practical importance in industry and in connection with foods. They are of equal scientific interest. Everyday experience shows that the phenomena of chronic intoxication are often quite distinct from those of acute poisoning by the same drugs. Notwithstanding this intrinsic interest, there has been relatively little experimental investigation of chronic poisoning; chiefly, no doubt, because of the difficulties of keeping and observing large numbers of animals for long periods under uniform conditions.

These difficulties appear to be largely removed by the discovery that white rats can be kept for their entire natural span of life on a uniform artificial diet. Under these conditions, their growth conforms closely to a standard curve. This is of very great importance in the study of the slowly progressive, and often rather indefinite symptoms that characterize chronic intoxications; for the growth may be considered as a cumulative index of *all* the effects produced upon the organism. The growth curve, especially of young animals, may therefore be taken for a primary criterion as to whether a drug produces chronic intoxication, and in which doses.

Unpoisoned rats thrive so normally under these artificial conditions, that the administration of the drugs can be continued for many months. This makes it possible to study the development of the intoxications when induced very slowly, i.e., it is possible, to reproduce the most distinctive feature of chronic intoxication. This duration may generally be made to approximate the actual duration of chronic intoxication in man. Moreover, the natural span of the life of rats is about three years; so that a year in its life would correspond in growth and development, to about twenty-five years in the life of man; or two weeks of the rat to a year of man.

The appetite of tame rats is apparently not easily influenced by the taste of drugs; in our experience, the taste must be much stronger than is likely to occur in chronic intoxications, before the appetite is at all checked; unless the drug has specific effects on growth.

The relatively low cost of the food and care of rats makes it possible to experiment on sufficient numbers to exclude accidental variations. For this, trouble may be saved by placing a group of animals in a cage, and treating the entire group as an average experiment. We started with groups of 10; but gradually descended to groups of three, as the most convenient experimental unit for simple experiments. Groups of six are, however, better if the time-factor and after-periods are to be studied. In that case, the whole group is placed on the poisoned food for, say, fifteen weeks. At that time, 2 of the group are killed for autopsy; 2 are continued on the poisoned food, and 2 are placed on normal food.

The details of care of the animals, will be described later.

NORMAL GROWTH CURVES

As it was planned to study the effects of the chronic intoxications mainly by the modifications of the growth, it became necessary to devise convenient means for comparing the growth of the poisoned rats with that of unpoisoned rats of the same weight. The growth charts of various observers are given by

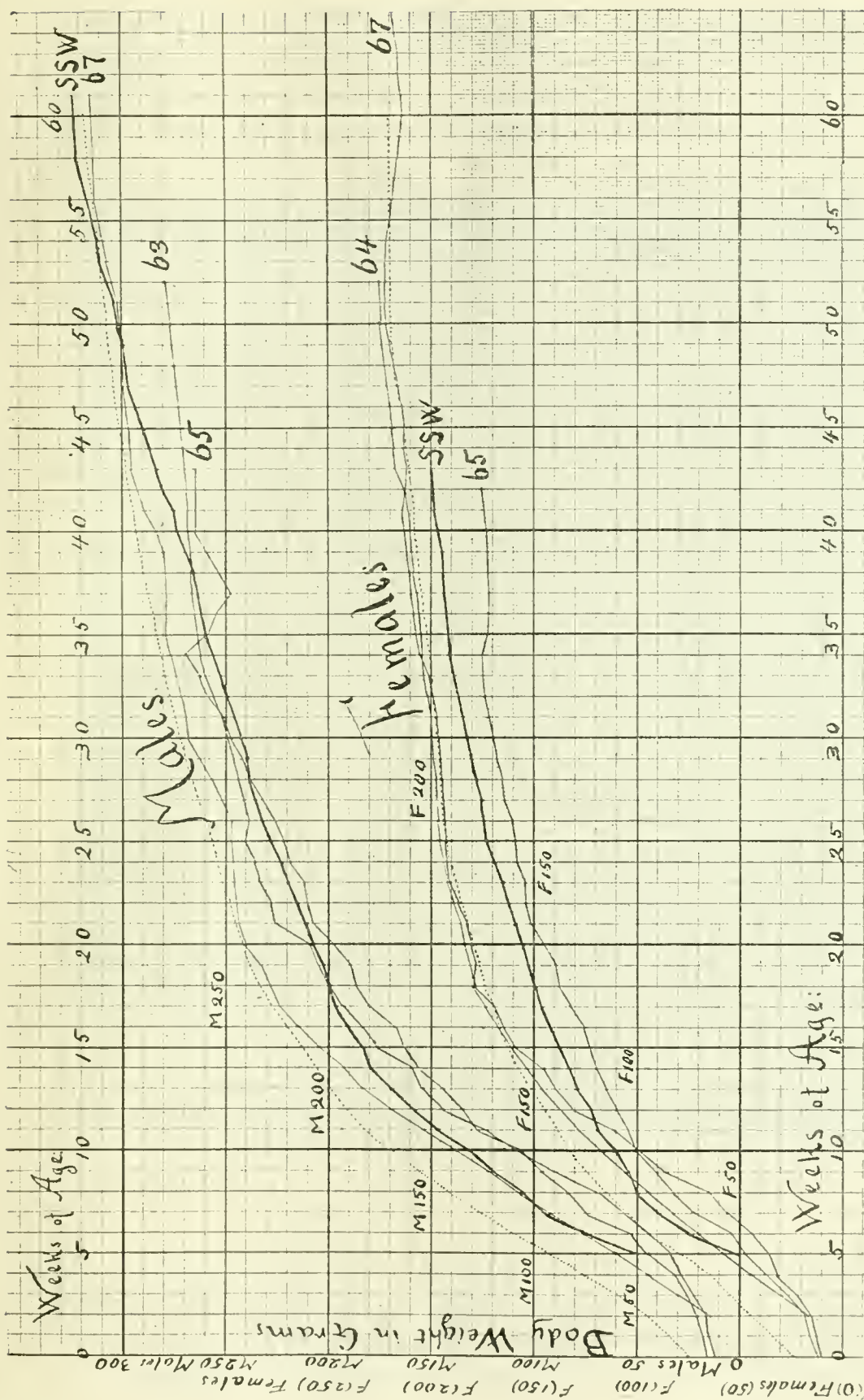


FIG. 1. GROWTH CURVES FOR ALBINO RATS

The curves *S.S.W.* are from the data of the authors. The numbered curves are transposed from the corresponding tables of Donaldson, and refer to the following originals. Table 62: Donaldson, Dunn and Watson, Chicago. Table 65: Ferry, New Haven. Table 67: King, Wistar.

The dotted line is that used in the standard growth charts.

Donaldson (1915). These were redrawn from his tables so as to be divided into weekly periods. We also drew an average curve from our earlier experiments. All these are shown in figure 1. The heavy line represents our own earlier data. It is

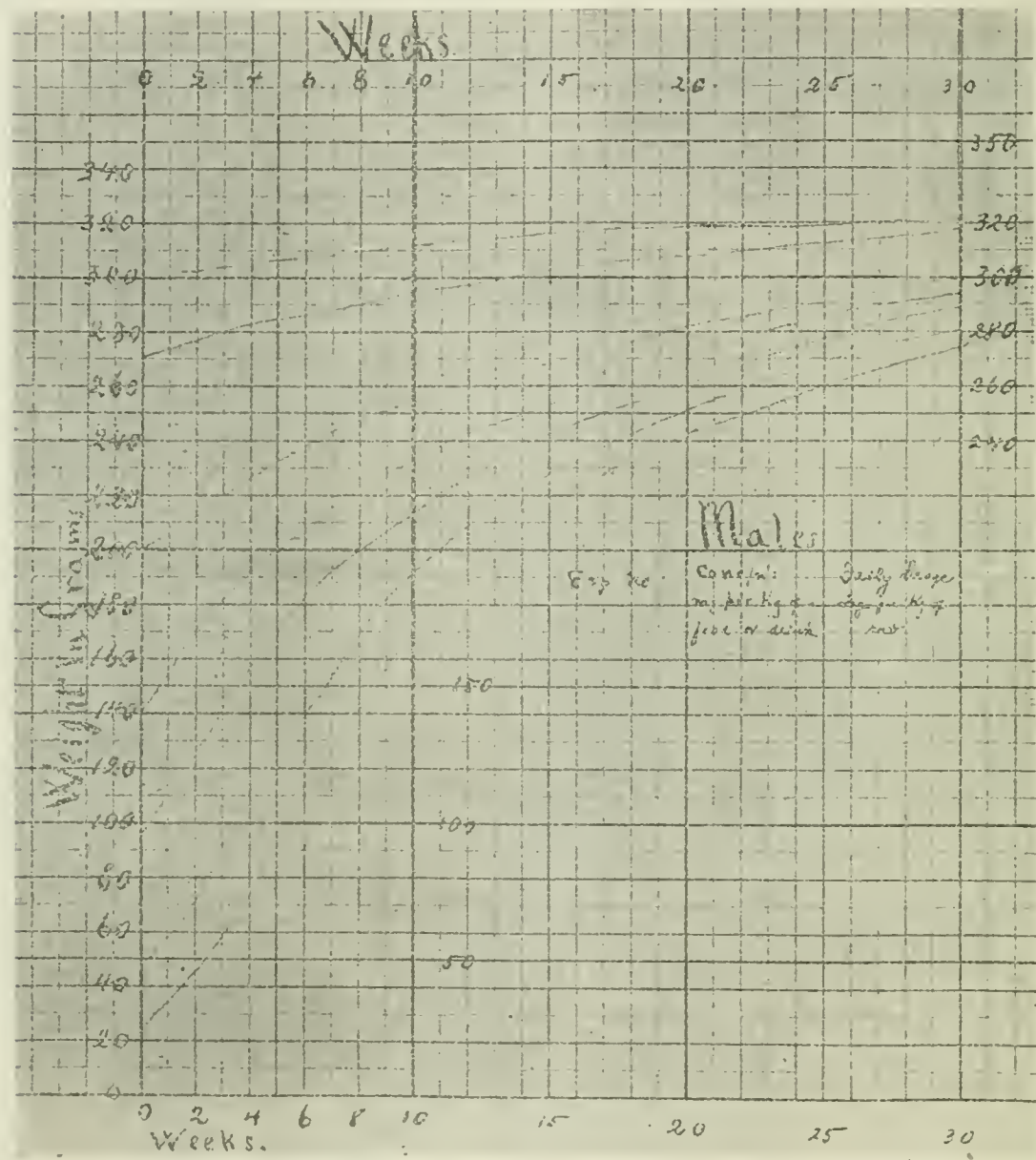


FIG. 2. STANDARD GROWTH CHARTS, MALE

a very satisfactory average of the data of the other investigators. Later experiments gave even better growth, approximating the dotted line, taken as our normal. This is an ideal curve, used in the construction of the standard normal growth charts.

It was convenient, and also more representative, to make experiments on rats of different ages and sizes, who therefore grew at a different rate. (Of course, all the rats that are treated as a single experiment must be of one sex and of at least approximately equal age.) Since the growth-rate varies with the age and sex, it would be necessary to plot for each experiment a normal growth curve for rats of the same initial weight. To avoid this trouble, and especially to make it possible to plot

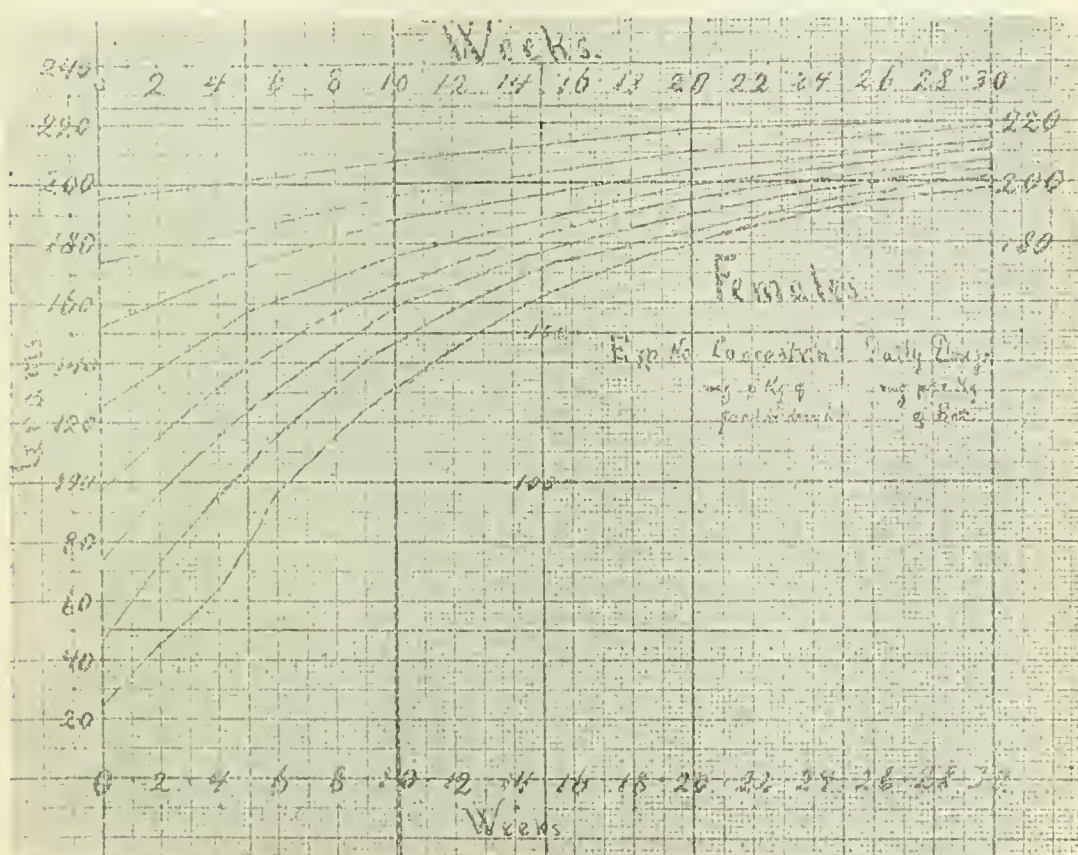


FIG. 3. STANDARD GROWTH CHARTS, FEMALE

animals of different size on one sheet, the normal growth curves for female rats of a starting weight of about 25, 50, 75, 100, and other multiples of 25 grams were drawn on one sheet, using the dotted curve of figure 1. Another similar sheet was made for male rats, as shown in figures 2 and 3. These were reproduced photographically (through the kindness of Prof. T. H. Todd); and the photographs were used as curve papers for the intoxication experiments.

Several experiments with unpoisoned food were always kept going, so as to control the possibility of accidental variations. Figures 4 and 5 reproduce these control curves. They illustrate the fair agreement of the growth with the standard curve.

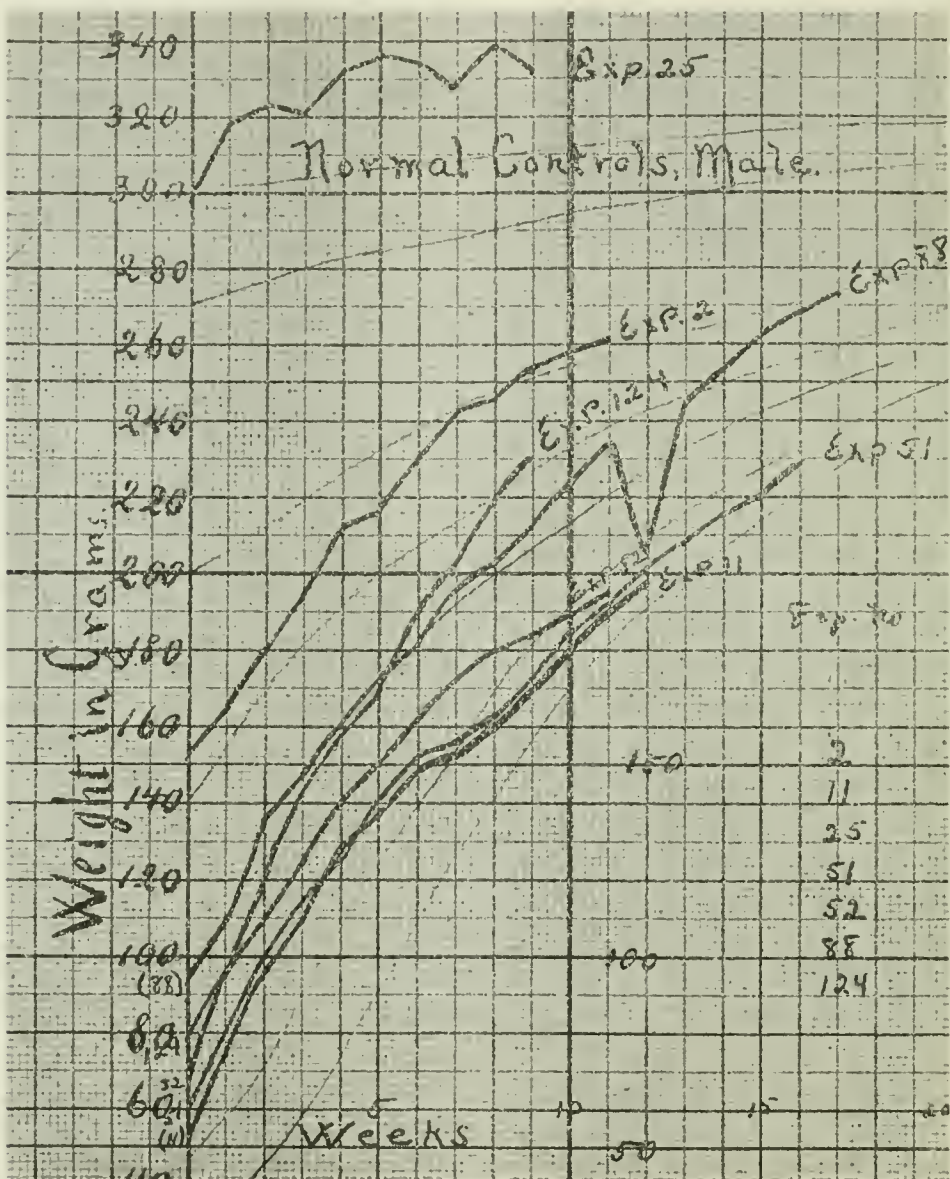


FIG. 4. ACTUAL GROWTH CURVES OF MALE RATS ON NORMAL FOOD

In order to permit a numerical comparison, the weight of the animals at the end of the observations was compared with that of the standard curves. The difference is expressed in percentage of the standard-weight; and this, divided by the number of weeks that the experiment has lasted, gives the average difference per week.

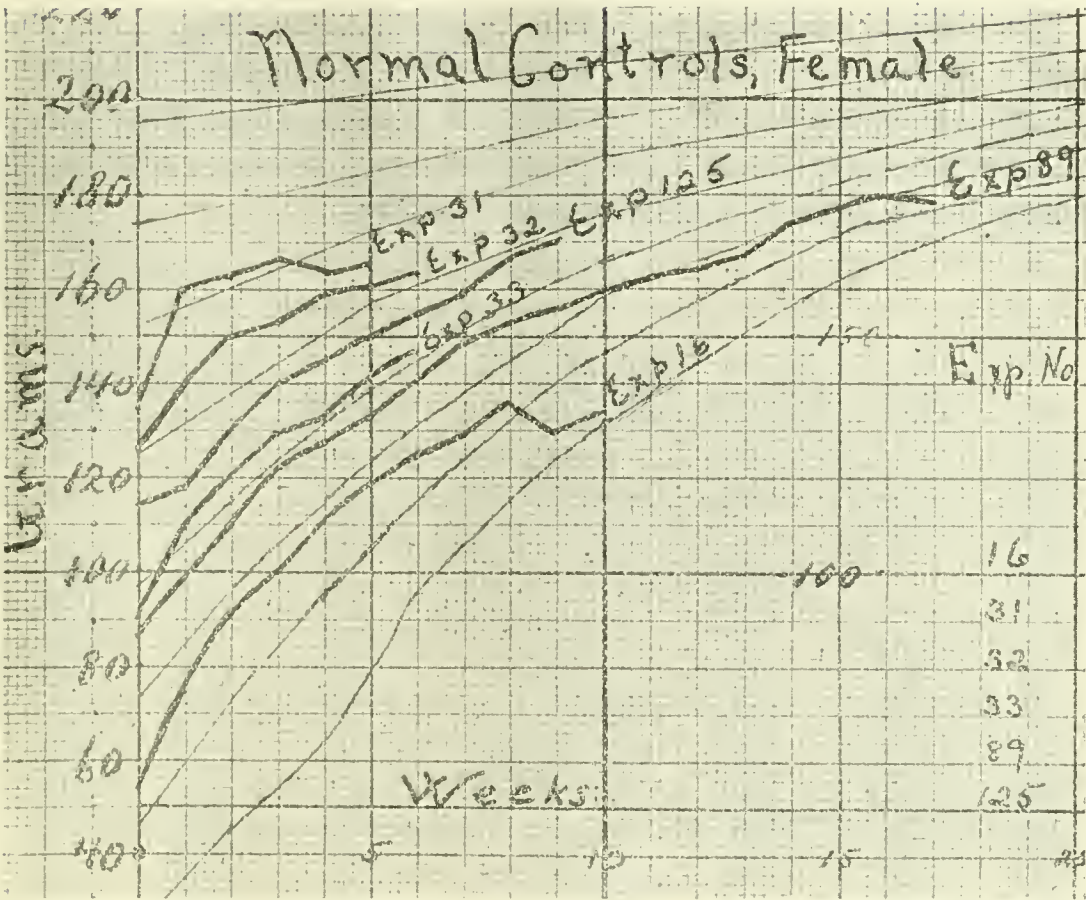


FIG. 5. ACTUAL GROWTH CURVES OF FEMALE RATS ON NORMAL FOOD

TABLE 1

Growth of unpoisoned rats; the data refer to the end of the experiments

EXPERI- MENT NUM- BER	SEX	OBSERVED WEIGHT	NORMAL WEIGHT	DIFFER- ENCE	DIFFERENCE AS PER CENT OF NORMAL WEIGHT	DURATION OF EXPERI- MENT	DIFFER- ENCE PER WEEK
		grams	grams	grams		weeks	per cent
51	M	229	236	-7	-2.9	16	-0.18
89	F	178	185	-7	-3.7	17	-0.21
52	M	197	204	-5	-2.2	11	-0.2
11	M	197	201	-4	-1.9	11	-0.17
32	F	162	161	+1	+0.62	6	+0.06
16	F	137	136	+1	+0.73	10	+0.07
125	F	169	167	+2	+1.2	9	+0.13
31	F	164	162	+2	+1.2	5	+0.24
33	F	147	138	+9	+6.5	6	+1.08
2	M	258	239	+19	+8.0	11	+0.73
88	M	273	243	+30	+12.3	17	+0.80
124	M	228	196	+32	+16.3	9	+1.8
25	M	330	308	+22	+7.1	9	+0.8

The control experiments, when figured on this basis, give the results shown in table 1. They show a very slightly better growth than the standard curve; the mean excess gain being 0.13 per cent per week. The greatest irregularities are in male rats of more than 225 grams, final weight.

TABLE 2
Standard food consumption of male rats

AGE	WEIGHT OF RATS	STANDARD FOOD CON- SUMPTION PER RAT PER DAY	FOOD PER KILOGRAM OF RATS PER DAY	AGE	WEIGHT OF RATS	STANDARD FOOD CON- SUMPTION PER RAT PER DAY	FOOD PER KILOGRAM OF RATS PER DAY
<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>weeks</i>	<i>grams</i>		<i>grams</i>
5	50	6.0	120	33	250	12.0	
6	75	7.4		34	255	12.1	
7	91	8.0		35	259	12.2	47
8	108	8.7		36	260	12.3	
9	120	9.0		37	262	12.3	
10	131	9.0	69	38	265	12.4	
11	145	9.4		39	270	12.5	
12	159	9.7		40	275	12.7	46
13	170	10.0		41	276	12.7	
14	178	10.1		42	279	12.9	
15	184	10.2	65	43	281	12.9	
16	190	10.3		44	286	13.0	
17	195	10.3		45	289	13.1	45
18	200	10.4		46	290	13.2	
19	202	10.4		47	292	13.2	
20	208	10.7	51	48	298	13.4	
21	210	10.7		49	299	13.4	
22	215	10.9		50	300	13.5	45
23	220	11.0		51	301	13.5	
24	221	11.0		52	305	13.6	
25	228	11.1	48	53	310	13.6	
26	230	11.1		54	312	13.6	
27	232	11.2		55	314	13.6	43
28	238	11.4		56	319	13.7	
29	240	11.4		57	320	13.7	
30	242	11.5	47	58	321	13.7	
31	245	11.6		59	322	13.8	
32	249	12.0		60	323	13.8	42

NORMAL FOOD CURVES

It is important to know whether changes in growth are paralleled by changes in food consumption. In order to obtain normal data, we averaged our standard food consumption data

from the earlier experiments. The results are shown in tables 2 and 3. In these, the age is computed from the weight.

In the individual experiments, the food data are plotted as crossed block graphs, on which the standard data appear as a dotted line. These graphs for the normal control experiments are shown in figures 6 and 7. They give very close agreement with the standard.

TABLE 3
Standard food consumption of female rats

AGE	WEIGHT OF RATS	STANDARD FOOD CON- SUMPTION PER RAT PER DAY	FOOD PER KILOGRAM OF RATS PER DAY	AGE	WEIGHT OF RATS	STANDARD FOOD CON- SUMPTION PER RAT PER DAY	FOOD PER KILOGRAM OF RATS PER DAY
<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>weeks</i>	<i>grams</i>		<i>grams</i>
5	50	6.0	120	25	173	9.3	53
6	72	7.1		26	175	9.3	
7	89	7.1		27	178	9.3	
8	100	7.1		28	179	9.3	
9	105	7.4		29	180	9.3	
10	111	7.7	69	30	182	9.3	51
11	120	7.7		31	186	9.3	
12	122	7.7		32	188	9.3	
13	129	7.7		33	189	9.3	
14	132	8.1		34	190	9.3	
15	139	8.4	60	35	191	9.3	48
16	142	8.6		36	192	9.3	
17	148	8.6		37	194	9.3	
18	150	8.6		38	194	9.3	
19	152	8.6		39	195	9.3	
20	157	9.0	57	40	198	9.3	46
21	160	9.0		41	198	9.3	
22	162	9.0		42	199	9.3	
23	166	9.3		43	200	9.3	
24	169	9.3					

In table 4, the food consumption is contrasted with the growth.

The mean difference in food consumption is an excess of 0.8 gram per rat per day, i.e., 7 per cent of the standard. The extremes range from 0.01 to 1.4 gram or -0.08 to $+19$ per cent.

The figures for the food and growth are generally parallel, but not strictly proportional. Taking the ratio of the food

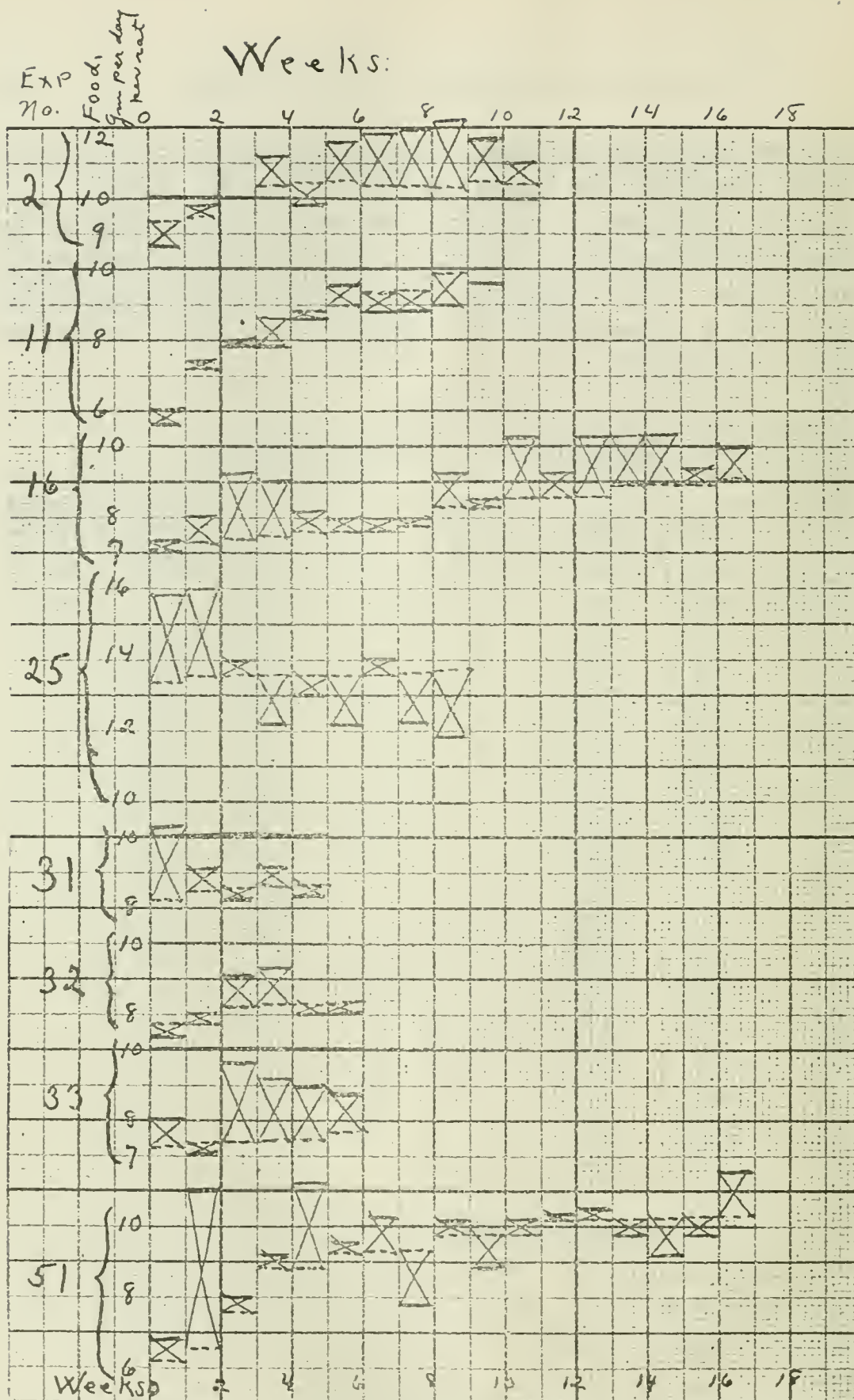


FIG. 6. FOOD CONSUMPTION

The actual consumption is shown in solid lines; the average consumption in dotted lines. The long line in each experiment corresponds to a daily food consumption of 10 grams of food per rat.

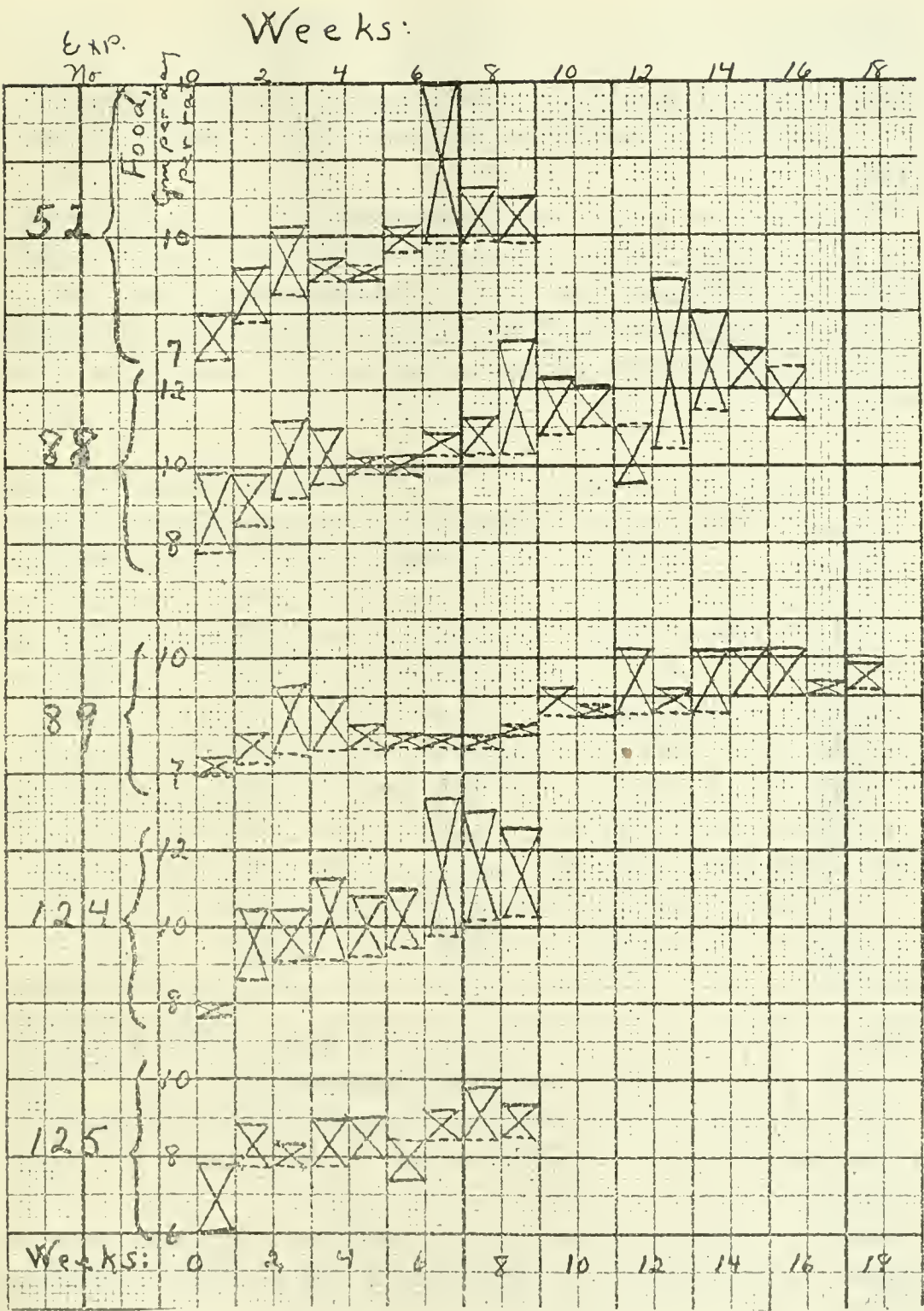


FIG. 7. FOOD CONSUMPTION—CONTINUED

difference to the growth difference, it appears that a difference of 0.8 gram of food (per rat per day) is equivalent, in the average, to a difference of 0.13 per cent per week in growth, i.e., as 6:1. Individual cases, however, sometimes differ much more widely.

Choice of food. Diminished food consumption raises the question whether this is due to diminished appetite in general, or to distaste for this particular food. This was tested by supplying the cage with 2 cups of food, one poisoned, the other unpoisoned; and noting whether these were consumed in the same ratio. The

TABLE 4
Food and growth of unpoisoned rats, mean difference from standard

EXPERIMENT	FOOD, GRAMS PER RAT PER DAY	FOOD, PER CENT DIFFERENCE FROM STANDARD	GROWTH, PER CENT PER WEEK	NUMBER OF RATS IN THE EXPERIMENT
2	+0.4	+3.0	+0.73	4
11	+0.2	+2.0	-0.17	10
16	+0.04	+0.4	+0.07	10
25	-0.01	-0.08	+0.8	10
31	+0.6	+7.0	+0.24	5
32	+0.2	+2.0	+0.06	5
33	+1.4	+19.0	+1.08	5
51	+0.4	+4.0	-0.18	6
52	+1.4	+15.0	-0.2	6
88	+1.3	+12.0	+0.8	2
89	+0.8	+9.0	-0.2	3
124	+1.5	+16.0	+1.8	3
125	+1.0	+13.0	+0.13	3

position of the two cups in the cage was shifted every day or so, to avoid the influence of accidental associations.

The same method was used to determine the development of "drug-habit," i.e., of preference for foods mixed with habit-forming narcotics.

Dosage. The experiments were conducted on the basis of definite concentrations of the drug in the food or drink; the absolute dosage depending upon the appetite and thirst of the animal. It is easily calculated from the data.

The starting dose may be planned on the basis that rats of 100 grams consume about 7.5 grams of food per day. A daily

dosage of 1 mgm. per kilogram of body weight of rat would therefore correspond to a concentration of about 13.3 mgm. per kilogram of food. A daily dosage of 1 mgm. per human would thus correspond to about 0.2 mgm. per kilogram of food. Conversely, a concentration of 1 mgm. per kilogram of food would correspond to a daily dosage of about $\frac{1}{13}$ mgm. per kilogram of rat; or 5 mgm. per human.

The dosage was then increased or decreased in subsequent experiments, according to effect; usually in geometrical ratio.

Size of rats. Effects on growth would be expected to be especially prominent with young animals. It was therefore aimed to use these, preferably of about 50 grams, at least in some of the experiments with each drug.

Sex. Female rats show a more uniform growth than males, and are therefore preferable.

Duration of the experiments. This varied according to circumstances but it was aimed to include for each drug some experiments lasting over twenty weeks.

After periods. In some experiments, especially those in which there was retardation of growth, some of the animals in each experiment were replaced on normal food, to study how they recovered from the effects of the drug. For comparison, a second subplot of the same group were killed and autopsied at once; and a third subplot were continued on the poison.

Autopsies. Nearly all animals were killed and autopsied by Dr. M. L. Richardson at the end of the experiment. Dr. Richardson is making a microscopic study of the tissues and will report his results in due time.

DETAILS OF THE CARE OF THE RATS

The preceding covers the general principles of the investigation. It remains to discuss the details of the care of the rats.

Cages. These could be comparatively simple, as it was not aimed to collect the excreta. The main desiderata were ample ventilation, compactness, and convenience in handling and cleaning. These were satisfactorily fulfilled by the type of cage that we devised.

Our cages consist of unpainted wooden 2 by 2-inch frames joined by $\frac{1}{4}$ -inch mesh galvanized wire netting, with a similar hinged drop-door in front. The rats are thus surrounded on four sides by air and light. This may perhaps help to explain the satisfactory health of the animals. The cages are five to seven stories high, the stories being separated by removable galvanized iron pans, two inches deep. These therefore form the ceiling and floor of the cages. For experiments with six to ten rats to the group, the individual sections are 42 inches deep, 13 inches high, 16 inches deep. For smaller groups we use cages with two sections to the story. The cages are mounted on castors, so that they are easily moved about the room. The room was well ventilated and adequately heated.

A thin layer of clean hard-wood sawdust was kept on the pans. Hard wood was chosen to avoid turpentine in ordinary sawdust—probably an unnecessary refinement. All the woodwork was unpainted, to avoid the introduction of paint poisons from gnawing of the wood.

The pans were cleaned once a week, at the time when the animals were weighed, the pans being merely rubbed down thoroughly with clean sawdust. Thanks to the excellent ventilation, this proved entirely adequate, as shown by the absence of odor, and especially by the excellent condition of the animals. We did not find it necessary to disinfect the cages at any time.

Diseases. The only outbreak of disease was a very mild epidemic of snuffles during November and December, affecting mainly our breeding animals. It was of short duration, and among the experimental animals was not sufficient to affect the growth or appetite.

Animals in poor condition, especially those in advanced phosphorus poisoning, sometimes developed a skin lesion, with small hemorrhagic ulcerations on the tail and ears, and somewhat also in the fur.

The autopsies confirmed that pulmonary disease proper was very rare, even in the older rats.

Food. We employed throughout a uniform diet of the Osborne-Mendel type. The “normal feed” consisted of:

	<i>per cent</i>
Skim milk powder ("Merrell-Soule").....	20
Gluten feed (18 to 22 per cent "protein").....	35
Corn starch.....	18
Sodium chlorid.....	1
Calcium carbonate.....	1
<hr/>	
Total ("dry feed").....	75
Mixed as needed with	
Leaf lard.....	20
Sweet butter.....	5
<hr/>	
Total.....	100

Different lots of "gluten feed" probably varied, and one lot was at first taken less readily; but the animals seemed to accustom themselves to it. However, we subsequently bought larger quantities so as to minimize the variations.

The feed was made up in two batches, namely: "Dry feed," in 60 kgm. lots, about every three weeks; and "normal feed," by the addition of the fats, in 20 kgm. lots, every week.

For the dry feed, the ingredients were mixed thoroughly in a large box of 100 kgm. capacity. Milk powder, when lumpy by exposure, was powdered by trituration and screened.

In mixing the normal feed, 1 kgm. of melted butter and 4 kgm. of melted lard were poured into 15 kgm. of the dry feed, and rubbed between the hands to a homogeneous mixture.

Poisoned feed. A concentrated stock mixture was made by rubbing the poison with successive portions of normal feed. The dilutions actually used were made fresh every week from this stock, by the gradual incorporation of the required quantities of normal feed.

Feeding cups. The feed was packed firmly into ordinary cylindrical tin "drinking cups" of 150 and 250 gram capacity which were then fitted with a concave top, provided with an inch hole in the center (the tops of tin "stencil pots"). This prevented spilling of the feed almost completely, even when the cup was upset. However, if the rats inclined to upset the cups, these were secured very simply by passing a wire rod through the handle of the cup and the meshing of the cage.

The cups were weighed with the feed, and at the end of the daily period, thus recording the quantity of food consumed. They were then refilled. Toward the end of the experiments, the weighing and refilling was done only every two or three days. This was more convenient and equally satisfactory. It is necessary, of course, that sufficient excess of food remain in the cups so that the rats can have ready access to it. With large animals, two cups were placed in the cage.

Additional food. Once per week, each cage received a small quantity of dry corn and fresh carrots; for ten rats, a handful of corn, and two good sized carrots, cut into cubes.

Water. This was furnished by 8 ounce bottles, fitted with a rubber stopper and glass tube. The inverted bottle was hung at the side of the cage so that the glass tube projected through the netting. The animals lap the water from the tube. The arrangement thus works automatically. The bottles were filled with fresh water daily, sometimes twice per day. Liquid drugs were added to this drinking water, and the daily consumption measured.

Weighing of the rats. This was done weekly. A separate record was at first kept for each rat; but later the group was weighed as a whole.

SUMMARY

Under the organization adopted, the growth of control rats differs from the "standard growth curve" by a range of -1 to $+1.8$ per cent per week, with a median difference of $+0.13$ per cent. Female rats show less variation than male rats.

Standards of food-consumption were determined; such that the extremes of control experiments differ from the standard by a range of 0.01 to 1.4 gram per rat per day, or -0.08 to $+19$ per cent; with a mean of $+0.8$ gram, or $+7$ per cent.

The departures from the standard curves are generally parallel for food and growth; but they are not strictly proportional.

Experiments results may be considered abnormal if

a. The growth differs from the standard by more than -1 and $+1.8$ per cent per week.

b. The food differs from the standard by more than -0.01 and 1.5 gram per rat per day; or by more than -0.08 and $+19$ per cent.

c. The growth and food depart from the standard in contrary directions.

REFERENCE

DONALDSON, H. H.: 1915 The Rat—Data and Reference Tables. Philadelphia.

STUDIES OF CHRONIC INTOXICATIONS ON ALBINO RATS

II. ALCOHOLS (ETHYL, METHYL AND "WOOD") AND ACETONE

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INTRODUCTION

Chronic intoxication by the alcohols has been so little studied experimentally, that further data are highly desirable. The peculiar contrast between the acute and subacute toxicity ratio of ethyl and methyl alcohol adds further interest to their behavior with really chronic intoxication. It has appeared desirable to ascertain whether chronic experiments would answer the question of the relative toxicity of pure methyl alcohol and commercial "wood alcohol," and incidentally of acetone.

The experiments were conducted as described in the first paper, using three to six rats for each experiment. The alcohols were added to the drinking water, so that they were consumed continuously.

Dosage of alcohol. This is determined by the concentration of the drug and by the thirst of the animals. The mean doses are shown in table 1. The weekly variations appear unimportant. They could be calculated from the curve of fluid consumption.

The dosage, in all cases, is fairly heavy; the larger doses approaching those ordinarily acutely fatal for dogs and rabbits.

For acute poisoning by stomach, Baer, 1898, places the minimum fatal dose at 7.2 to 9 grams of methyl alcohol, and 6.25 to 7.44 grams of ethyl alcohol, per kilogram of rabbit.

TABLE 1
Mean dosage of alcohol, and duration of experiments

CONCENTRATION OF ALCOHOL	EXPERIMENT NUMBER	NUMBER OF ANIMALS IN EXPERIMENT	DURATION	DOSAGE OF ALCOHOL PER KILOGRAM OF RATS PER DAY
			<i>weeks</i>	<i>cc.</i>
Ethyl, 2.5 per cent.....	103	3	18	2.7 (1.2- 3.7)
Ethyl, 5 per cent.....	4798	4	21	4.8 (3.6- 6.4)
Ethyl, 5 per cent.....	75	3 to 6	19	4.7 (1.8- 6.8)
Ethyl, 5 per cent.....	130	3	8	6.4 (5.2- 7.0)
Ethyl, 10 per cent.....	110	3	10	9.9 (8.6-10.9)
Ethyl, 10 per cent past 4 weeks of 5 per cent.....	7594	3	15	8.9 (5.8-13.5)
Methyl, 2.5 per cent.....	100	3	18	2.6 (1.8- 3.4)
Methyl, 2.5 per cent.....	129	3	9	3.6 (3.2- 4.7)
Wood, 2.5 per cent.....	6599	3	21	2.8 (2.2- 3.7)
Wood, 2.5 per cent.....	101	2	18	3.6 (2.4- 7.4)
Acetone, 2.5 per cent.....	102	3	18	1.8 (1.4- 2.2)
Acetone, 2.5 per cent.....	139	3	2	3.1 (3.1- 3.2)
Methyl, 5 per cent.....	86	5	6	3.1 (2.3- 3.2)
Methyl, 5 per cent.....	109	3	3	3.5 (3.3- 4.9)
Methyl and wood, 5 per cent....	76	4	5	5.4 (4.1- 6.0)
Wood, 5 per cent.....	87	3	6	3.3 (2.5- 5.2)

TABLE 2
Mean daily consumption of fluid

ALCOHOLS	EXPERIMENT NUMBER	DURATION	FLUID CONSUMPTION PER KILOGRAM OF RAT			
			Entire period	First third	Middle third	Last third
		<i>weeks</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
Ethyl, 2.5 per cent.....	103	18	110 (48-150)	131	109	107
Ethyl, 5 per cent.....	4798	21	95 (72-129)		101	95
Ethyl, 5 per cent.....	75	19	93 (37-136)	122	89	86
Ethyl, 5 per cent.....	130	8	127 (105-140)	134	127	125
Ethyl, 10 per cent.....	110	10	99 (86-109)	107	99	88
Ethyl, 10 per cent.....	7594	15	89 (58-135)	124	85	100
Methyl, 2.5 per cent.....	100	18	103 (72-136)	95	116	93
Methyl, 2.5 per cent.....	129	9	143 (126-188)	161	150	141
Wood, 2.5 per cent.....	6599	21	111 (89-147)		109	115
Wood, 2.5 per cent.....	101	18	145 (95-296)	102	144	147
Acetone, 2.5 per cent.....	102	18	72 (56-89)	80	70	68
Acetone, 2.5 per cent.....	139	2	125 (122-127)			
Methyl, 5 per cent.....	86	6	62 (45-63)	49		62
Methyl, 5 per cent.....	109	3	69 (66-97)			
Wood and methyl, 5 per cent...	76	5	103 (81-119)	116		87
Wood, 5 per cent.....	87	6	65 (50-104)	53		76

For repeated, daily administrations, Hunt, 1902, found death to follow after three doses of 5 grams of methyl alcohol per kilogram of dog, or eight doses of 2.5 grams methyl alcohol per kilogram of rabbit (after seventeen days), i.e., a dosage approximating that which was fatal to the rats in this series. Ethyl alcohol was not fatal to rabbits after four doses of 6 grams per kilogram, but killed after six such doses. Dogs however, increased in weight after five doses of 6 grams. Our rats also tolerate this dose without trouble.

Consumption of fluid. This would presumably be affected most directly by the alcohol. Table 2 shows the mean daily consumption of fluid in each experiment; as well as the mean for the first, middle and last third of each experiment so as to indicate the influence of the continuance of the experiment.

A study of table 2 shows the following:

Ethyl alcohol. There is no material difference between the fluid consumption when this fluid contains 2.5 or 5 or 10 per cent of ethyl alcohol. In every experiment, the fluid consumption is greatest in the first third of the experiment; declines sharply in the middle third and then remains about constant in the last third. The mean of all the experiments faithfully repeats the individual experiments. It is as follows.

ENTIRE DURATION	FIRST THIRD	MIDDLE THIRD	LAST THIRD
97	123	100	97

The falling off after the first third is perhaps due to diminished muscular activity of the alcoholized rats, but we have no direct observations on this point.

Methyl and wood alcohol, 2.5 per cent. These have about the same mean consumption as ethyl alcohol, or rather larger. The most characteristic difference is, however, that the fluid consumption generally increases in the middle third, instead of diminishing, as with ethyl alcohol. The mean of all the experiments are:

ENTIRE DURATION	FIRST THIRD	MIDDLE THIRD	LAST THIRD
127	102	130	128

The increase in the first third is perhaps due to habituation to the taste of methyl and wood alcohol. Accordingly, the increase is greater with the wood-alcohol with which the taste is more pronounced.

Acetone, 2.5 per cent. In the one experiment with this drug that lasted sufficiently long to justify conclusions, the fluid consumption was markedly below the normal. The data resemble those of 5 per cent wood alcohol.

They are as follows:

ENTIRE DURATION	FIRST THIRD	SECOND THIRD	LAST THIRD
72	80	70	68

Methyl and wood alcohol, 5 per cent. These showed a marked decrease from the start and persisting to the early death.

ENTIRE DURATION	FIRST THIRD	SECOND THIRD	LAST THIRD
67	53		76

EFFECTS ON GROWTH

These are shown in table 3 and figures 1 to 3. In the table, data are given for the end of the experiments, and for other periods when important changes occurred in the curve.

Ethyl alcohol. With the exception of one experiment (no. 130) all the concentrations showed defective growth approaching or exceeding the extreme normal deficit (-1 per cent per week). The mean deficit for each concentration is

CONCENTRATION	DOSE	DURATION	WEEKLY DEFICIT
<i>per cent</i>	<i>cc.</i>	<i>weeks</i>	<i>per cent</i>
2.5	2.7	18	1.2
5.0	4.8	19	1.0
10.0	9.4	13	1.8

Consequently, 2.5 and 5 per cent ethyl alcohol or 2.7 and 4.8 cc. per kilogram per day, interfere quite considerably with growth, and to about equal degrees. Ten per cent alcohol, or 9.4 cc. per kilogram per day, produces more marked interference.

TABLE 3
Effects of alcohols and acetone on growth

DRUG AND CONCENTRATION	DOSAGE PER KIL- OGRAM OF RAT PER DAY	EXPER- IMENT NUM- BER	DURA- TION	OB- SERVED WEIGHT	NOR- MAL WEIGHT	DIFFER- ENCE	DIFFER- ENCE AS PER CENT OF NORMAL WEIGHT	DIFFER- ENCE PER WEEK
	cc.		weeks	grams	grams	grams		per cent
Ethyl alcohol, 2.5 per cent.....	2.7	103	18	168	220	-52	-23.0	-1.2
Ethyl alcohol, 5 per cent.	4.8	4798	21	215	279	-64	-23.0	-1.0
Ethyl alcohol, 5 per cent.	4.7	75	19	192	250	-58	-23.0	-1.2
Ethyl alcohol, 5 per cent.	6.4	130	8	204	200	+4	+2.0	+0.25
Ethyl alcohol, 10 per cent.	9.9	110	10	194	220	-26	-11.8	-1.1
Ethyl alcohol, 10 per cent after 4 weeks of 5 per cent.....	8.9	7594	15	155	244	-89	-36.0	-2.4
Methyl alcohol, 2.5 per cent.....	2.6	100	18	232	248	-16	-6.0	-0.3
Methyl alcohol, 2.5 per cent.....	3.6	129	8	122	200	-78	-39.0	-5.0
Wood alcohol, 2.5 per cent.....	2.8	6599	{ 6	178	202	-24	-11.8	-1.9
			{ 18	155	260	-105	-41.0	-2.3
			{ 20	163	268	-105	-40.0	-2.0
Wood alcohol, 2.5 per cent	3.6	101	{ 7	240	340	-100	-29.0	-4.1
			{ 18	252	336	-84	-25.0	-1.4
			{ 2	227	229	-2	-0.9	-0.4
Acetone, 2.5 per cent.....	1.8	102	{ 3	154	229	-75	-32.0	-11.0
			{ 18	150	230	-80	-34.0	-1.9
Acetone, 2.5 per cent.....	3.1	139	2	109	100	+9	+9.0	+4.5
Methyl alcohol, 5 per cent	3.1	86	{ 2	120	160	-40	-25.0	-12.5
			{ 6	88	200	-112	-56.0	-9.3
Methyl alcohol, 5 per cent.....	3.5	109	3	203	245	-42	-13.0	-4.0
Methyl and wood alcohol, 5 per cent.....	5.4	76	5	84	204	-20	-9.0	-1.8
Wood alcohol, 5 per cent.	3.3	87	6	58	156	-98	-62.0	-10.0

Methyl and wood alcohol, 5 per cent. These behave alike. In dosages corresponding to 3.1 to 5.4 cc. per kilogram per day, they produce a very considerable loss of weight, starting

within the first week of administration, and continuing until death, which occurs in a few weeks. The median weekly retardation of growth is 9 (1.8 to 12.5) per cent. The total actual loss was 17 to 51, mean 37 per cent. The loss is therefore very much greater than with 10 per cent ethyl alcohol.

Methyl and wood alcohol, 2.5 per cent. These behave alike. All experiments show considerable deficits in growth, and often

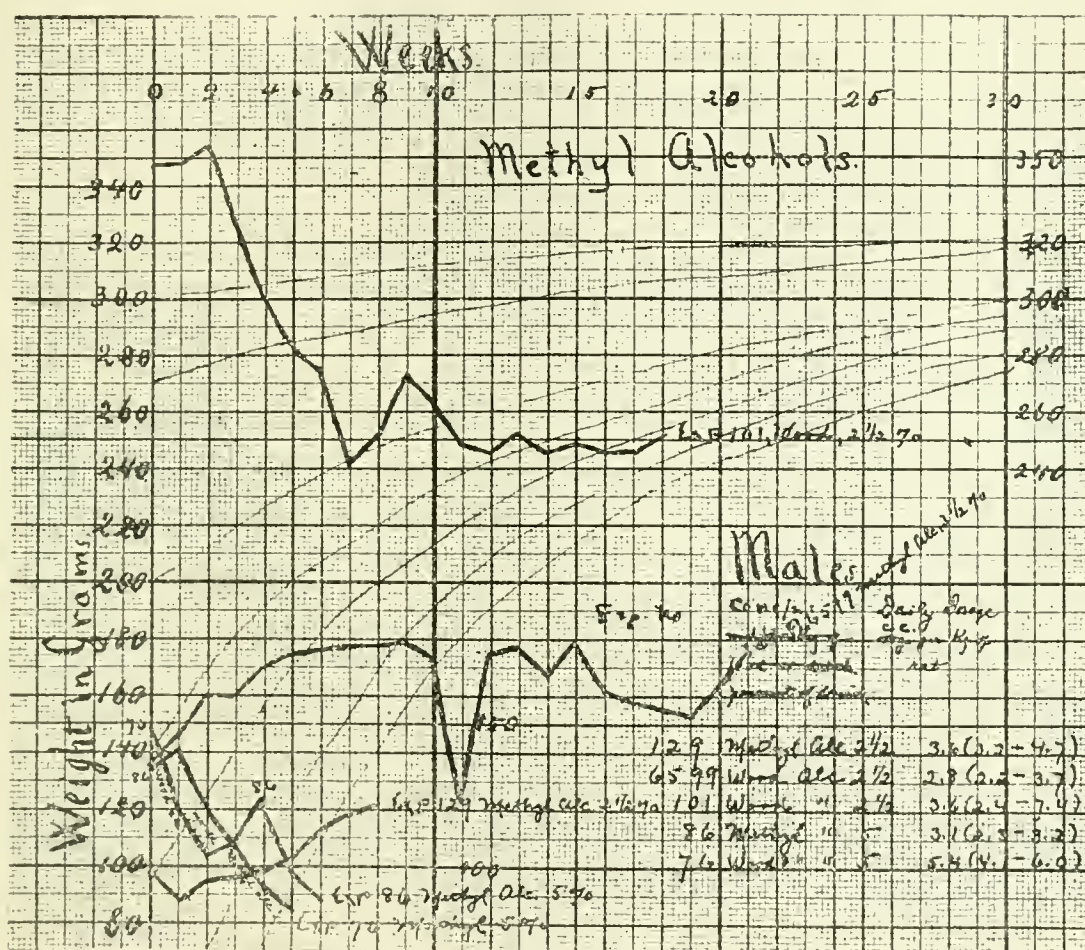


FIG. 3. METHYL AND WOOD ALCOHOLS ON GROWTH OF MALE RATS

actual loss of weight; but the extent of the growth-deficit varies widely, from 0.3 to 5 per cent per week, with a mean of -2 per cent. The observations include four experiments, with seven periods; and a mean dosage of 3.2 cc. per kilogram per day.

The largest differences were in two groups of extra large adult rats. One of these (100) had a deficit of only -0.3 per cent per week; whereas the other (101) lost 32 per cent of its original

weight between the second and the seventh week, and then remained almost stationary. This was evidently mainly a result of dosage; for table I shows that the rats of experiment 100 drank relatively lightly, while those of experiment 101 drank quite heavily, especially in the earlier periods.

In the other experiments, the retardation of growth began with the start of the experiment, and continued steadily to the end, with a tendency to keep all growth in abeyance.

The growth-interference of 2.5 per cent methyl and wood alcohol (3.2 cc. per kilogram per day) is therefore on the whole distinctly more serious than with 10 per cent ethyl alcohol (9.4 cc. per kilogram per day).

Acetone, 2.5 per cent. The one experiment (102), that is sufficiently prolonged (eighteen weeks, with mean of 1.8 cc. per kilogram per day) shows a final loss (1.9 per cent) similar to 2.5 per cent methyl and wood alcohol.

However, the form is peculiar, in that a large loss occurs between two and three weeks (32 per cent of the weight), after which the weight remains stationary. The effect resembles that of experiment 101 with 2.5 per cent wood alcohol; except that the acetone loss is more abrupt.

In principle, then the effect of acetone is allied to that of wood alcohol of the same concentration and somewhat higher dosage, but is more severe.

It should be noted that the large loss of weight cannot be attributed to nausea from the taste of the acetone or wood alcohol; for the loss starts only after two weeks.

EFFECTS ON FOOD CONSUMPTION

These are shown in table 4 and figures 4 to 8.

In interpreting the data of table 4, it must be remembered that the extreme variation of unpoisoned rats from the standard food averages lies between -0.01 and $+1.4$ gram per rat per day or -0.08 to $+19$ per cent. Practically every experiment with all the alcohols, shows a much greater deficit than the extreme for the normal experiment. In most of the alcohol experiments,

the food consumption fails to increase, as it should in normally growing animals, but remains level, or declines. There is also a frequent tendency for temporary anorexia; the food consumption being much lower on some days than on others. These sudden changes are often reflected in the growth curve. Generally they go hand in hand, i.e., the food- and growth-curves are generally parallel; but sometimes (as in experiment 101, methyl alcohol) the

TABLE 4
Food consumption

ALCOHOLS	EXPERI- MENT NUMBER	DURATION OF EXPERI- MENT	GROWTH, MEAN DIFFER- ENCE FROM NOR- MAL STAN- DARD, PER WEEK	FOOD CONSUMPTION, MEAN DIFFERENCE FROM NORMAL STANDARD PER RAT PER DAY		MEAN DIFFER- ENCE IN FOOD FOR SERIES
				grams	per cent	
		<i>weeks</i>	<i>per cent</i>			<i>per cent</i>
Ethyl, 2.5 per cent.....	103	18	-1.2	-1.6	-17	-17
Ethyl, 5 per cent.....	4798	21	-1.0	-1.9	-17	-10
Ethyl, 5 per cent.....	75	19	-1.2	-1.0	-10	
Ethyl, 5 per cent.....	130	8	+0.25	+0.9	+10	
Ethyl, 10 per cent.....	110	10	-1.1	-2.0	-19	-23
Ethyl, 10 per cent.....	7594	15	-2.4	-2.9	-27	
Methyl, 2.5 per cent.....	100	18	-0.3	-0.3	-3	
Methyl, 2.5 per cent.....	129	9	-5.0	-4.7	-50	-28
Wood, 2.5 per cent.....	6599	21	-2.0	-3.2	-30	
Wood, 2.5 per cent.....	101	18	-1.4	-3.6	-26	
Acetone, 2.5 per cent.....	102	18	-1.9	-2.15	-23	-23
Acetone, 2.5 per cent.....	139	2	+4.5	+0.5	-7	-52
Methyl, 5 per cent.....	86	6	-9.3	-7.0	-71	
Methyl, 5 per cent.....	109	3	-4.0	-3.4	-36	
Wood and methyl, 5 per cent.....	76	5	-1.8	-6.0	-59	
Wood, 5 per cent.....	87	6	-10.0	-3.6	-45	

loss of appetite sets in *after* the loss of weight. This favors the view that the appetite does not control the weight, but that both are controlled by a common cause, i.e., the general health of the animal.

Ethyl alcohol. Table 4 and figure 4 show that 2.5 per cent alcohol interferes seriously with appetite, the daily deficit averaging 17 per cent. With 5 per cent alcohol, the mean deficit happens

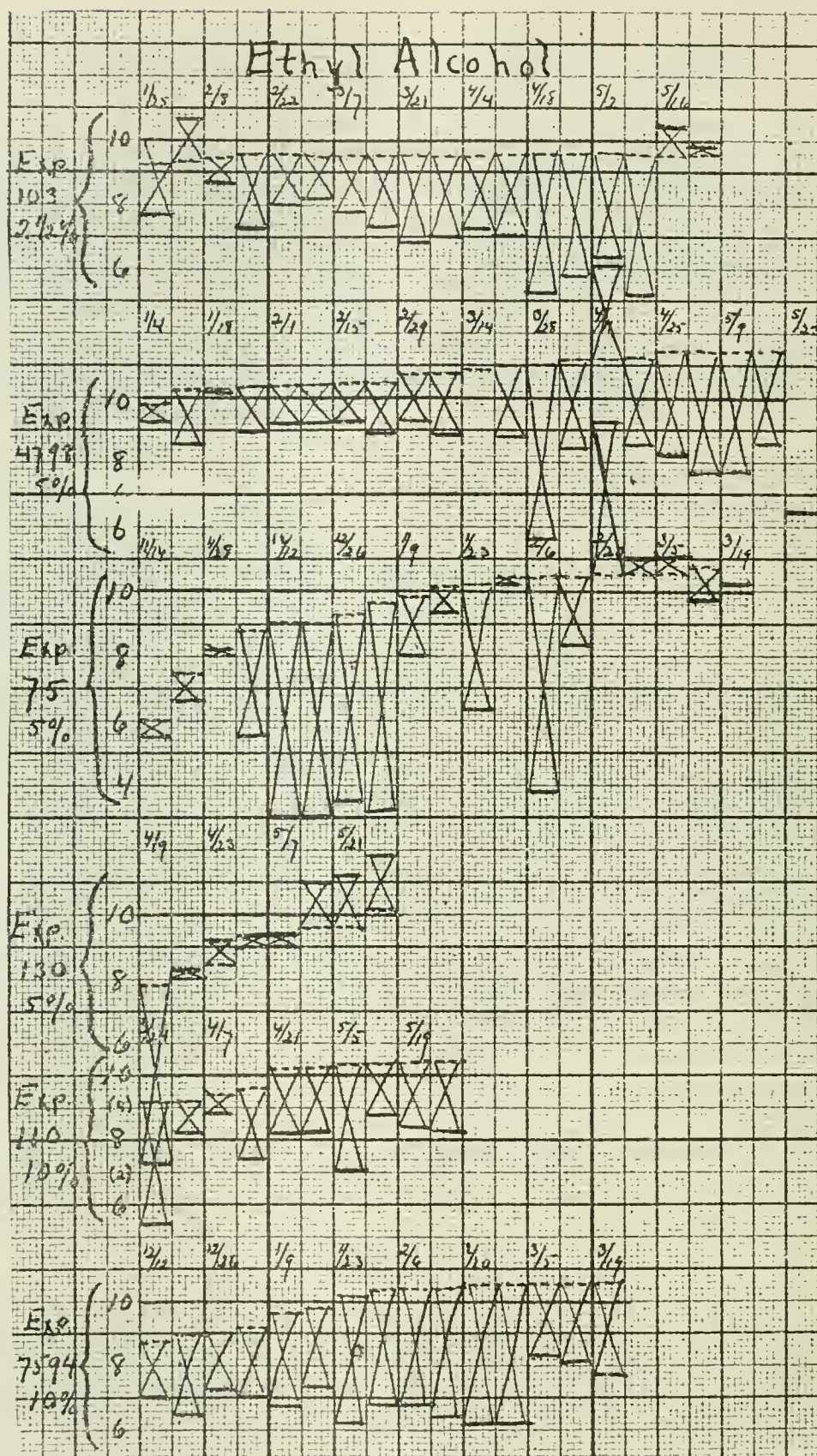


FIG. 4. ETHYL ALCOHOL ON FOOD CONSUMPTION

The numbers to the left represent grams of food consumed daily per rat. The numbers above each experiment are the dates of the observations. The dotted horizontal lines represent the standard food consumption; and the solid horizontal lines joined to the dotted lines by crossed lines represent the actual food consumption.

Methyl and Wood Alcohols, 2 1/2%

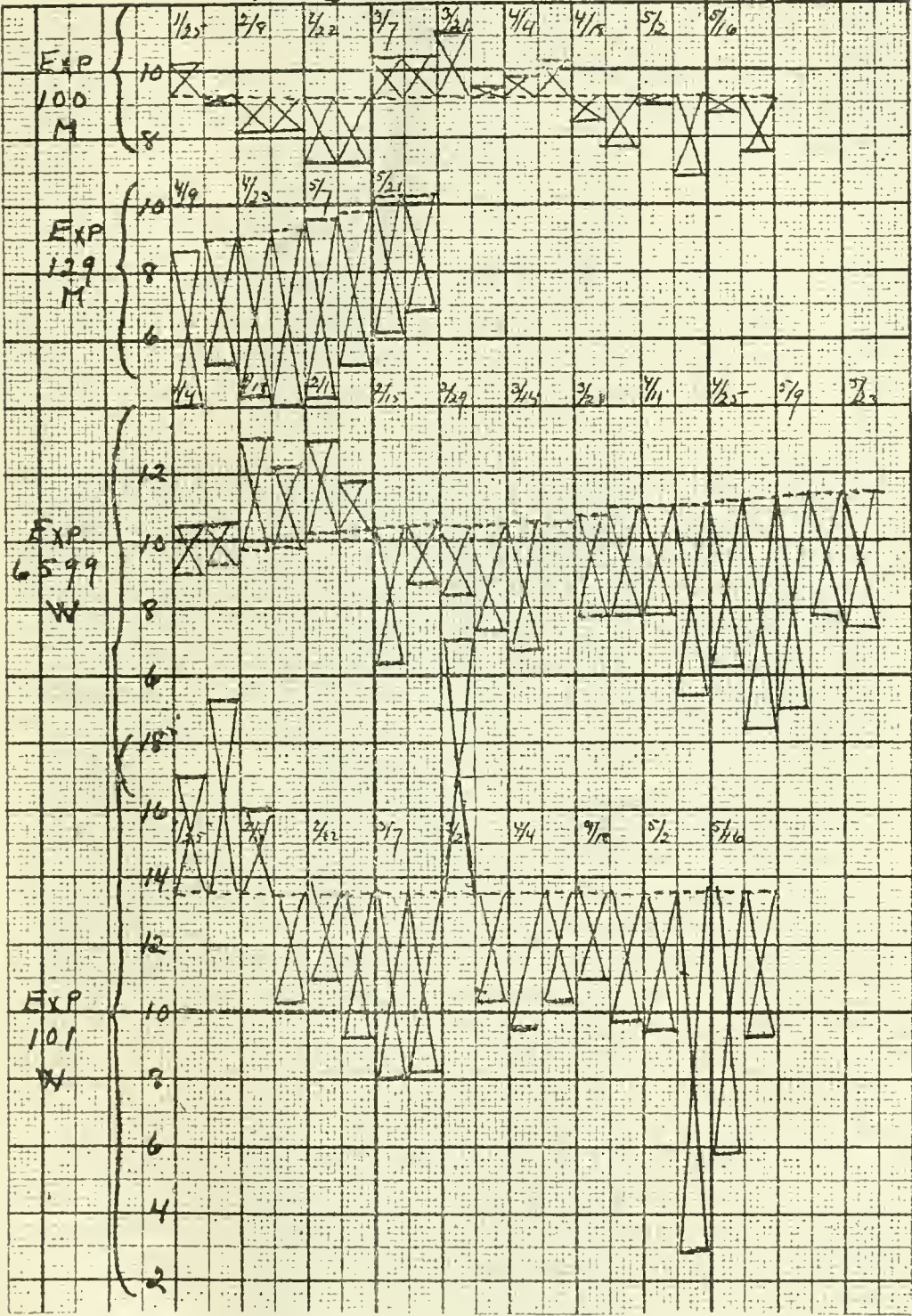


FIG. 5. METHYL AND WOOD ALCOHOLS, 2.5 PER CENT, ON FOOD CONSUMPTION

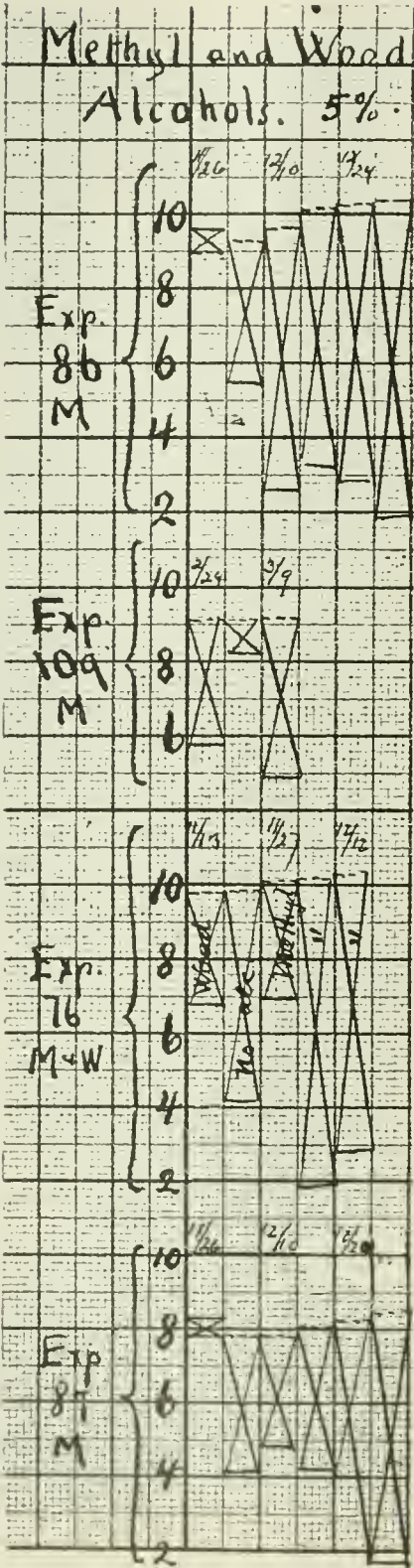


FIG. 6. METHYL AND WOOD ALCOHOLS, 5 PER CENT ON FOOD CONSUMPTION

to be a little smaller, (10 per cent; range +10 to -17); with 10 per cent alcohol, the deficit is considerably greater (-19 to -27, mean -23 per cent).

Methyl and wood alcohol. These behave alike. The deficit with 2.5 per cent (-3 to -50; mean -28 per cent) is greater than with 10 per cent ethyl alcohol (fig. 5). With 5 per cent methyl and wood alcohols the deficit is very marked (-36 to -71, mean -52 per cent).

Acetone. With the one good 2.5 per cent experiment, the food deficit (-23 per cent) is that of 10 per cent ethyl alcohol. The sharp decline in the body weight in the third week of experiment 102 is reflected in the appetite of the same week.

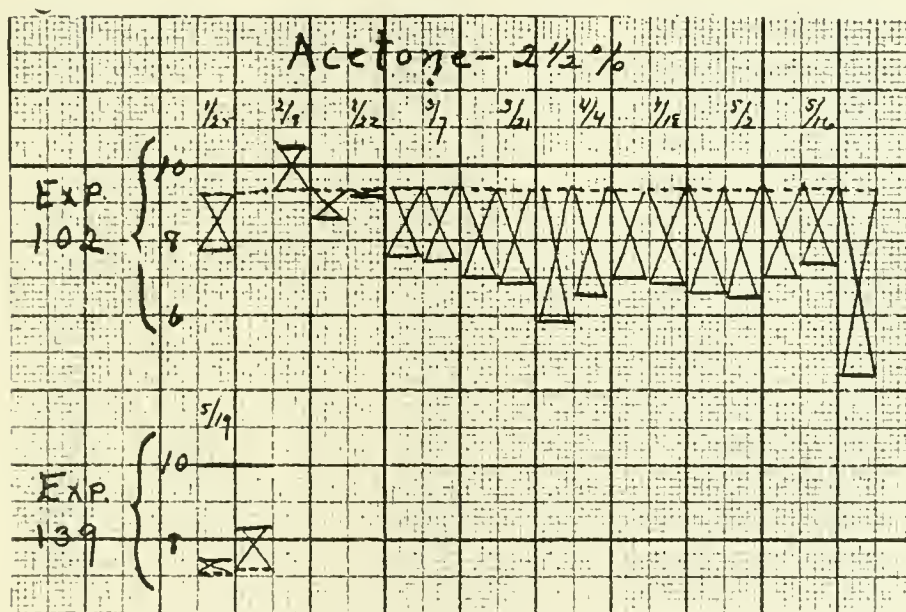


FIG. 7. ACETONE ON FOOD CONSUMPTION

MORTALITY

This is shown in table 5 for the individual experiments, and is summarized for each alcohol in table 6.

Since the duration of the alcohol administration is doubtless a very important factor in the mortality, the last column of table 6 divides the total mortality by the duration of the experiment. This factor is evidently the best measure for comparing the relative toxicity of the alcohols.

The actual sequence of this factor and its relation to the actual dosage is shown in table 7.

This table shows that 2.5 per cent alcohol and 2.5 per cent acetone can be consumed daily for over eighteen weeks, in daily doses corresponding to 2.8 cc. of absolute alcohol, or 1.8 cc. of acetone, per kilogram of rat, without mortality.

TABLE 5

Mortality

DRUG AND CONCENTRATION	DOSAGE	EXPERIMENT NUMBER	NUMBER OF ANIMALS	WEEKS OF FATALI- TIES	TOTAL DURATION	PER CENT OF FATALITIES
					<i>weeks</i>	
Ethyl alcohol, 2.5 per cent.	2.7	103	3	0	18	0
Ethyl alcohol, 5 per cent...	4.8	4798	4	0	21	0
Ethyl alcohol, 5 per cent...	4.7	75	6	1, 13	19	33 $\frac{1}{3}$
Ethyl alcohol, 5 per cent...	6.4	130	3	0	8	0
Ethyl alcohol, 10 per cent..	9.9	110	3	0	10	0
Ethyl alcohol, 10 per cent after 4 weeks of 5 per cent.....	8.9	7594	3	13	15	33 $\frac{1}{3}$
Methyl alcohol, 2.5 per cent.....	2.6	100	3	6	18	33 $\frac{1}{3}$
Methyl alcohol, 2.5 per cent.....	3.6	129	3	0	8	0
Wood alcohol, 2.5 per cent.	2.8	6599	3	0	20	0
Wood alcohol, 2.5 per cent.	3.6	101	2	0	18	0
Acetone, 2.5 per cent.....	1.8	102	3	0	18	0
Acetone, 2.5 per cent.....	3.1	139	3	0	2	0
Methyl alcohol, 5 per cent.	3.1	86	5	3, 4	6	40
Methyl alcohol, 5 per cent.	3.5	109	3	2, 3	3	66 $\frac{2}{3}$
Methyl and wood alcohol, 5 per cent.....	5.4	76	4	2, 2, 5, 5	5	100
Wood alcohol, 5 per cent...	3.3	87	3	7, 7	6	66 $\frac{2}{3}$

With the following (doses = cubic centimeters of absolute per kilogram of rat per day) the mortality is so low that it may be accidental: Ethyl alcohol 5 per cent (4.8 cc.), methyl and wood alcohol, 2.5 per cent (3.2 cc.) and perhaps ethyl alcohol 10 per cent (9.4 cc.).

Severe fatality occurs within two to eight weeks with 5 per cent wood alcohol (3.3 cc.) and 5 per cent methyl alcohol (3.5 cc.); the methyl alcohol being distinctly more toxic than the wood alcohol.

TABLE 6
Mortality summarized

DRUG AND CONCENTRATION	DOSAGE OF ALCOHOLS, DAILY MEDIAN CUBIC CENTI- METERS PER KILO- GRAM	WEEKS	MORTALITY RATIO	MORTALITY PER CENT	MORTALITY FACTOR $\frac{M\%}{\text{WEEKS}}$
Ethyl alcohol, 2.5 per cent....	2.7	18	0:3	0	0
Ethyl alcohol, 5 per cent.....	4.8	$\left\{ \begin{array}{l} 8 \\ 13 \text{ and } 19 \end{array} \right.$	$\left\{ \begin{array}{l} 1:13 \\ 2:10 \end{array} \right.$	$\left\{ \begin{array}{l} 8 \\ 20 \end{array} \right.$	$\left\{ \begin{array}{l} 1 \\ 1 \end{array} \right.$
Ethyl alcohol, 10 per cent....	9.4	$\left\{ \begin{array}{l} 10 \\ 15 \end{array} \right.$	$\left\{ \begin{array}{l} 0:6 \\ 1:3 \end{array} \right.$	$\left\{ \begin{array}{l} 0 \\ 33 \end{array} \right.$	$\left\{ \begin{array}{l} 0 \\ 2\frac{1}{3} \end{array} \right.$
Methyl and wood alcohol, 2.5 per cent.....	3.2	$\left\{ \begin{array}{l} 15 \\ 16 \\ 18 \end{array} \right.$	$\left\{ \begin{array}{l} 0:11 \\ 1:11 \\ 1:8 \end{array} \right.$	$\left\{ \begin{array}{l} 0 \\ 9 \\ 13 \end{array} \right.$	$\left\{ \begin{array}{l} 0 \\ \frac{2}{3} \\ \frac{2}{3} \end{array} \right.$
Acetone, 2.5 per cent.....	1.8	18	0:3	0	0
Methyl alcohol, 5 per cent....	3.5	$\left\{ \begin{array}{l} 2 \\ 3 \\ 4 \\ 5 \end{array} \right.$	$\left\{ \begin{array}{l} 3:12 \\ 5:12 \\ 6:12 \\ 8:12 \end{array} \right.$	$\left\{ \begin{array}{l} 25 \\ 40 \\ 50 \\ 67 \end{array} \right.$	$\left\{ \begin{array}{l} 12\frac{1}{2} \\ 13\frac{1}{3} \\ 12\frac{1}{2} \\ 13\frac{1}{2} \end{array} \right.$
Wood alcohol, 5 per cent.....	3.3	$\left\{ \begin{array}{l} 6 \\ 7 \end{array} \right.$	$\left\{ \begin{array}{l} 0:3 \\ 2:3 \end{array} \right.$	$\left\{ \begin{array}{l} 0 \\ 67 \end{array} \right.$	$\left\{ \begin{array}{l} \\ 9\frac{1}{2} \end{array} \right.$

TABLE 7
Mortality and dosage

CONCENTRATION	DRUG	MORTALITY PER WEEK	MORTALITY PER CC. PER KILOGRAM PER DAY
<i>per cent</i>		<i>per cent</i>	
2.5	Ethyl alcohol	0	0
2.5	Acetone	0	0
2.5	Methyl and wood alcohol	$\frac{2}{3}$	0.22
5.0	Ethyl alcohol	1	0.21
10 0	Ethyl alcohol	$2\frac{1}{3}$	0.24
5.0	Wood alcohol	$9\frac{1}{2}$	2.9
5.0	Methyl alcohol	$13\frac{1}{2}$	4.1

Symptoms. These were observed only superficially.

Ethyl alcohol. The rats showed no effect with 2.5 per cent; with 5 per cent the animals were a trifle "wild," i.e., shy and easily frightened. With 10 per cent, they were slightly "doped" (stupid) and wild.

Methyl and wood alcohol. Two and one-half per cent, were slightly doped and with roughened fur. With 5 per cent, they were more markedly doped, weak, with eyes half closed.

Acetone. Two and one-half per cent were practically normal.

Effect of concentration on the toxicity of methyl alcohol. The great difference in mortality between 2.5 and 5 per cent methyl alcohol does not depend on a difference in the mean daily dosage, in terms of absolute methyl alcohol. This is practically alike for both concentrations, because the rats drank only half as much of the 5 per cent as of the 2.5 per cent alcohol. This reduction in fluid consumption started with the first day, in the experiment (109) in which a daily record of the first week was kept, so that the greater mortality is not explained by excessive dosage on the first day or week. It appears, therefore, that the same dosage of methyl alcohol is more toxic, in chronic experiments, when taken in 5 per cent than when taken in 2.5 per cent concentrations. This might be explained by diminished fluid and food consumption, which probably causes damage that adds itself to that produced by the methyl alcohol directly. This appears more plausible than the alternative explanation of differences in the rate of absorption: for even if they existed, they would not be expected to play an important part in chronic poisoning.

SUMMARY

Groups of rats received for drink exclusively the various diluted alcohols, for periods up to twenty-one weeks (five months).

Dosage. The mean daily consumption, cubic centimeters of absolute alcohol per kilogram of animals, ranged for the various groups as follows:

	cc.	<i>The equivalent dosage for a 60 kg. man would be</i>
Ethyl alcohol, 2.5 per cent.....	2.7	160 cc.
Ethyl alcohol, 5 per cent.....	4.8	290 cc.
Ethyl alcohol, 10 per cent.....	9.4	560 cc.
Methyl* and wood alcohol 2.5 per cent.....	3.2	190 cc.
Methyl and wood alcohol 5 per cent.....	3.4	200 cc.
Acetone, 2.5 per cent.....	1.8	110 cc.

* The methyl alcohol was the Baker brand, 95 per cent; N. V. M., 0.002 per cent; C_3H_6O , trace.

Consumption of fluid. The addition of ethyl alcohol to the drink diminished the consumption of fluid by about a fifth; possibly by diminishing the activity of the animals. There was no material difference between the various concentrations and doses used.

Methyl and wood alcohols, 2.5 per cent, at first decreased the consumption of fluid about a fifth due perhaps to the taste; but this was only temporary, the ultimate consumption being rather more than with ethyl alcohol.

With 5 per cent, these alcohols very greatly decreased the fluid consumption (by nearly half) from the start, until the early death.

Acetone, 2.5 per cent caused considerable decrease, of fluid consumption (by about a third) through the eighteen weeks of the experiment.

Growth. Ethyl alcohol interfered with the growth of the animals. The interference was considerable with 2.5 and 5 per cent; and more marked with 10 per cent.

Methyl and wood alcohols, 5 per cent, produced very considerable loss of weight (by about one-third) starting with the first week of the administration, and continuing until death, which occurred in a few weeks.

Methyl and wood alcohols and acetone, 2.5 per cent, behaved alike, and showed considerable deficits in growth, varying widely in different experiments but generally distinctly more severe than with 10 per cent ethyl alcohol; though much less than with the 5 per cent methyl alcohols.

Food consumption. Ethyl alcohol definitely diminished the consumption of food, by about one-tenth with 2.5 and 5 per cent alcohol; and by about one-fourth with 10 per cent alcohol.

Methyl and wood alcohol (and acetone 2.5 per cent) again behaved alike. The deficit was considerably greater than with ethyl alcohol; namely nearly a third for 2.5 per cent; and about half for 5 per cent.

Relation of food consumption and growth. These generally showed parallel changes, although not always in strict proportion. As the change in growth may precede the change in food consumption, the diminished appetite is evidently not the cause of the checked growth. It may be either the effect; or more likely, diminished growth and diminished appetite are both results of a common cause.

Mortality. Ethyl alcohol and acetone, 2.5 per cent may be consumed for over four months as exclusive drink and in the large dosage of this experiment without fatality.

Ethyl alcohol, 5 per cent; and methyl and wood alcohol 2.5 per cent have a slight mortality (one per cent or less per week of duration) that might be accidental.

Ethyl alcohol, 10 per cent has a higher fatality ($2\frac{1}{3}$ per cent per week) but the number of animals was limited, so that this also could have been accidental.

Methyl and wood alcohol 5 per cent have a high and prompt mortality, viz., about 10 per cent per week of duration; the methyl being distinctly more toxic than the wood alcohol.

The greater toxicity of methyl alcohol in 5 per cent concentration is probably due to the additive damage of voluntary restriction of liquid and food consumption.

CONCLUSIONS

The continuous consumption by rats of ethyl alcohol, in doses of 2.7 to 9.4 cc. per kilogram per day, interferes considerably with their growth, and diminishes the consumption of food. Little or no mortality occurs even after periods of months.

Methyl and wood alcohols and acetone are markedly more toxic than ethyl alcohol. Methyl and wood alcohol 3.4 cc. per kilo-

gram per day as 5 per cent solution produced very great loss of body weight; greatly diminished consumption of food and drink, and caused death within a few weeks. "Wood alcohol" is rather less toxic than pure methyl alcohol. The same dose, in more dilute form, i.e., as 2.5 per cent solution produced very few fatalities even after prolonged administration. Their effects on growth and food consumption are more deleterious than those of ethyl alcohol in three times the dosage.

Acetone, 1.8 cc. per kilogram per day as 2.5 per cent, is not fatal even after four months administration. Its effects on growth and food consumption are about the same as 2.5 per cent methyl alcohol.

The investigation brings out the deleterious effects of chronic alcoholism on growth.

It emphasizes that the dangers of chronic alcoholism are much greater with methyl than with ethyl alcohol.

It proves that the "impurities" of wood alcohol play only a minor part in chronic intoxication, the methyl alcohol itself being the dominant toxic agent.

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THE EFFECT OF CARMINATIVE VOLATILE OILS ON THE MUSCULAR MOVEMENTS OF THE INTESTINE

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The volatile oils are widely used as carminatives, but the manner in which they produce carminative effects is not well understood. If one consults the various text books on pharmacology it is evident that a difference in opinion exists as to what type of change the volatile oils in therapeutic doses produce in the muscular movements of the alimentary canal. Thus, some authors say that the volatile oils produce relaxation of the gastric and intestinal musculature (1) and must therefore allow escape or onward progression of gases or contents passively. Others convey the opposite impression and state positively or as probable that carminatives increase the muscular movements of the alimentary canal (2). Muirhead and Gerald (3) found that dilute solutions of volatile oils, e.g., 1:50,000, produced increase in tone and in the amplitude of the rhythmic contractions in isolated pieces of intestine immersed in suitable oxygenated solutions, while stronger solutions, e.g., 1:5000, caused relaxation. Some of the volatile oils they used belong to the carminative group, others do not.

The volatile oils when used as carminatives, are mild remedies and must exert their primary effect on the mucous membranes of the alimentary canal; ordinarily the dosage is small and the concentration of the drug as it is applied is relatively low. It seems quite probable that their effects might easily be obscured by experimental conditions; that the results of their application to isolated pieces of intestine where they reach all surfaces in

equal concentration, as in Muirhead and Gerald's experiments, or when their action is tested in intact animals where other disturbing factors are present such as anaesthesia and operative procedures, may fail to give a true picture of what happens when they are applied to mucous membranes in dilute solution.

These considerations made it seem worth while to investigate the effects of volatile oils on the intestinal movements in experiments where no anaesthetic was used, where no operative procedures were necessary at the time of the experiment and where they could be applied to the mucous membrane in concentrations comparable with those employed therapeutically.

METHODS

The experiments were carried out on two dogs with Thiry-Vella loops. Both were young females of a large breed and weighed from 12 to 14 kgm. The loops were formed from the ileum and were between 45 and 50 cm. in length. The skin wound at the lower openings of the loop was rather slow in healing in these dogs but both were ready for use in the experiments in two or three weeks. The muscular movements of the isolated loops were recorded by means of a toy balloon attached by a rubber tube to a Brodie bellows recorder. The balloons used were of the "sausage" type about 15 mm. in diameter by 100 to 120 mm. in length. The small rubber tube to which the balloon was attached extended inside the balloon to its blind end and this portion of the tube had numerous side openings. In the early experiments air transmission was used from balloon to bellows; later the balloon and its rubber tube were filled with water and attached to an upright tube which exerted a water pressure of 25 to 35 cm. in the balloon, with air transmission from the top of the upright column to the bellows recorder. The latter method of recording the muscular contractions was found to be very satisfactory. These dogs were very easily trained to lie quietly during an experiment. The animal was placed on its side in a comfortable position on a padded table, and by having an assistant stroke its head, it usually slept

throughout the one to two and one-half hours that the experiment lasted, without any sedative or anaesthetic whatever. The balloon was placed in the loop by first passing two small elastic catheters, tied together, through the loop from the upper to the lower opening, the balloon tube was then tied to the catheter and the balloon drawn gently through the lower opening into the loop. Fluids were injected into the loop through a small catheter that was passed along side the balloon tube so that its end was near the upper part of the balloon. Fluids introduced into the loop were always at body temperature and were injected slowly so as to prevent distension of the loop. The quantities injected and the rate of injection were such as had been found to cause no appreciable change in the tracing when plain water or salt solution were introduced. Only dilute solutions of the various volatile oils were used because it was desired to find out what sort of action they had when employed in concentrations comparable with those used therapeutically. For the most part the volatile oils were employed in the form of the official "waters," i.e., saturated aqueous solutions filtered clear by the use of talcum. They were injected either undiluted or after dilution with several volumes of water. The spirits of the volatile oils (i.e., 10 per cent solutions in alcohol) were also used after diluting them with from 5 to 20 volumes of water, the dilution being made just before the injections.

Respiratory movements do not record on the tracing unless they are very violent as in sneezing or howling, and even in these cases the waves produced are entirely different from those of muscular contractions in the loop. The great majority of the experiments were performed eighteen to twenty-two hours after feeding, but in the few experiments carried out two or three hours after a meal the results were the same as in the fasting dog. The loops in the two dogs gave practically identical tracings of muscular movements; there has been no appreciable change in the reactions of the loops during the three to five months since they were made. They reacted alike to volatile oils.

RESULTS

The results summarized below are based on 55 experiments in which more than a hundred applications of volatile oils were made; the results were clear cut and very uniform as far as their general characteristics are concerned.

Tone and rhythmic contractions. The primary effect is an increase in tone of the intestinal wall as shown by a rise in the "diastolic" level of the tracing, and an increase in the extent of the rhythmic contraction as shown by the height of the waves on the tracing. Usually both tone and rhythmic contractions were increased at the same time (figs. 1 and 2); sometimes,

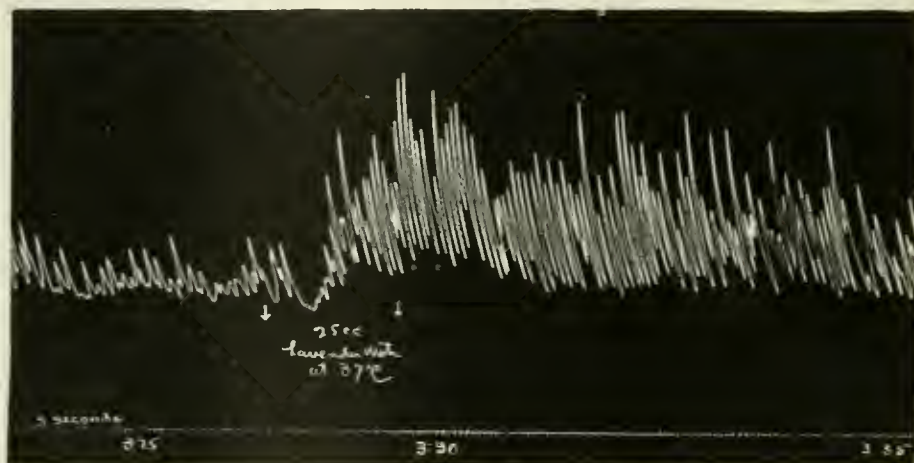


FIG. 1. DOG "L." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. 25 cc. lavender water introduced into loop between the arrows.

especially where the augmentation in tone was very marked the height of the rhythmic waves was not immediately increased, the increase in amplitude appearing as the tone subsided (fig. 3). The condition of the loop as regards tone did not modify the result; the tone of the loop varied somewhat from day to day, but regardless of whether the tracing showed only the rhythmic contractions or both rhythmic waves and recurring tonus waves, the application of volatile oils produced the same kind of effects. These changes are present when dilute solutions are injected just as when stronger ones are employed, e.g. diluted cinnamon water compared with spirit of cinnamon diluted 1:10 (figs. 4 and 5).

The effects of diluted solutions were studied in two ways, (a) starting with the undiluted "water" and in succeeding appli-

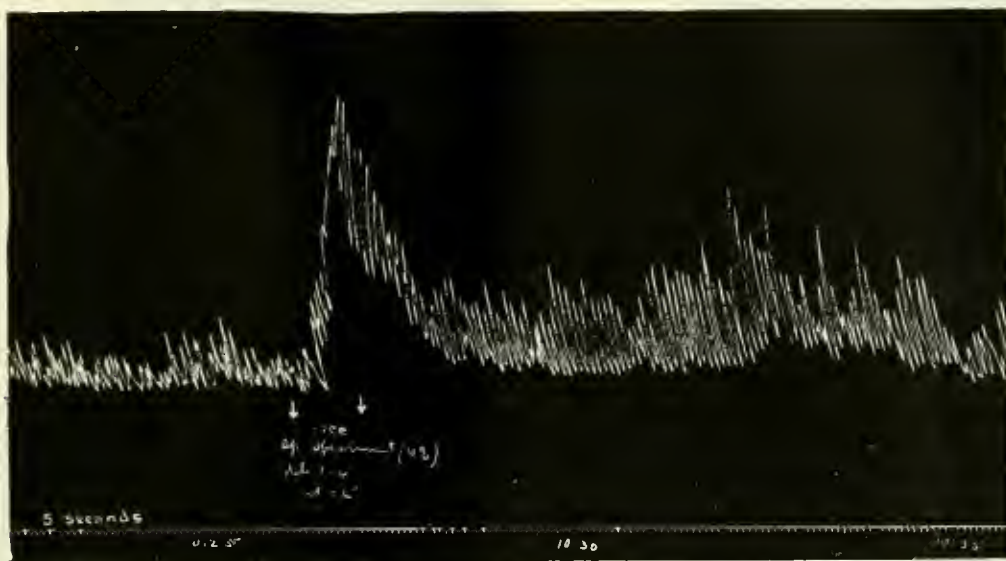


FIG. 2. DOG "L." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. 10 cc. spirit of spearmint, diluted 1:5, introduced into loop between the arrows.

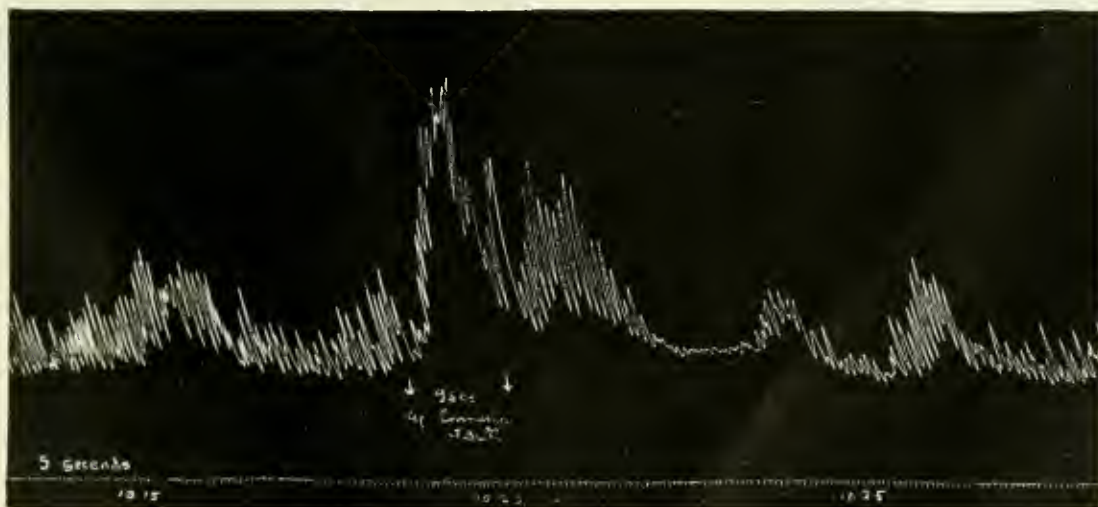


FIG. 3. DOG "L." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. 25 cc. cinnamon water introduced into loop between the arrows.

cations increasing the dilution until no effect was obtained and (b) by starting with dilutions that gave no result and increasing the concentration until an effect was produced. In both cases



FIG. 4. Dog "G." BALLOON FILLED WITH AIR

Small bellows recorder. Fast drum. 25 cc. diluted (1:20) cinnamon water introduced into the loop between the arrows.

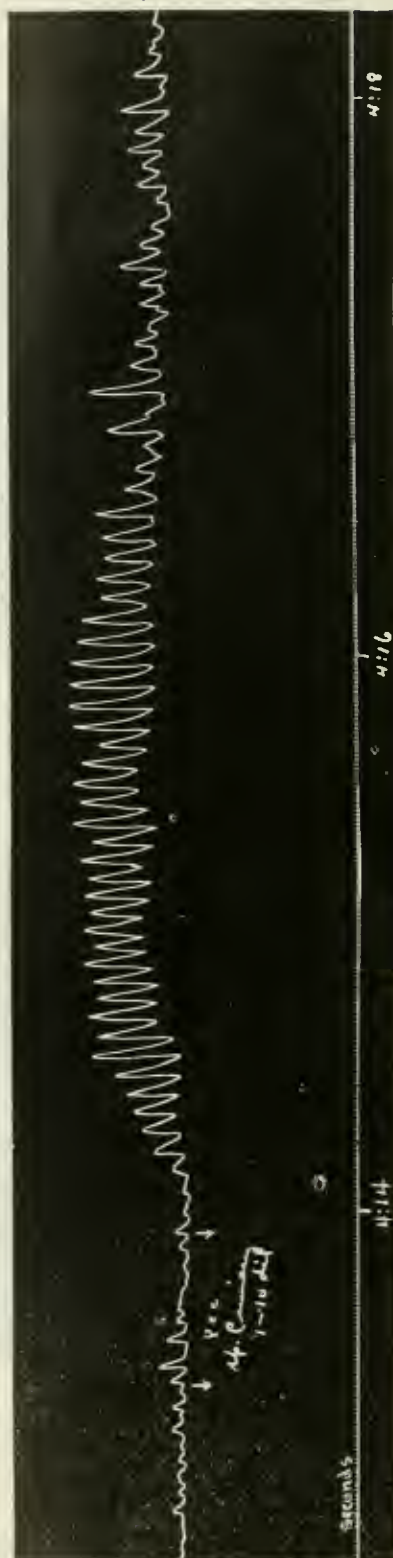


FIG. 5. Dog "G." BALLOON FILLED WITH AIR

Small bellows recorder. Fast drum. 8 cc. spirit of cinnamon, diluted 1:10, introduced into loop between the arrows.

it was found that peppermint or cinnamon water produced the changes above described when diluted with 5 to 20 parts of water (figs. 4 and 6).

As a rule this primary effect was not followed by any after effect, the tracing gradually returning to normal after the solution had escaped from the lower opening of the loop as in figure 3; occasionally, however, the period of stimulation was followed by one of decrease in tone and in amplitude of the rhythmic waves which lasted for a variable time (fig. 6). This after effect did not occur with any degree of regularity; even in the same experiment it usually failed when the same dose was applied

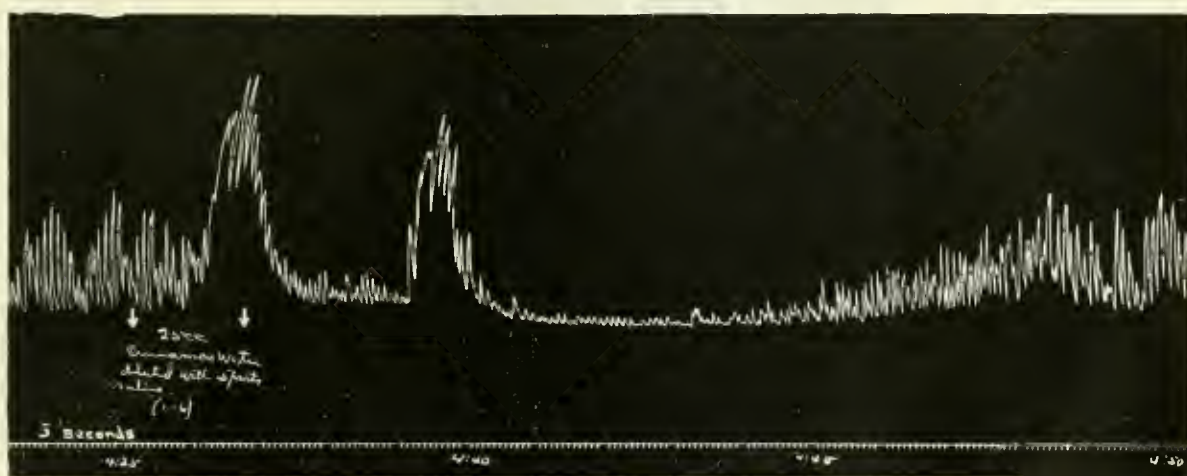


FIG. 6. DOG "L." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. 25 cc. diluted (1:6) cinnamon water introduced into loop between the arrows.

a second time, or it may have been absent after the first application and only appeared after the second or third injection. When a dilute solution (e.g., cinnamon water diluted 1:5) was injected slowly and continuously for twenty to thirty minutes the typical tonus waves and increase in amplitude were maintained throughout the injection, without any decrease occurring as an after effect (fig. 7).

Peristalsis. The question as to whether true onward peristalsis is affected during the action of the volatile oils can not be answered by inspection of the tracings made in the type of experiment described above. In an attempt to interpret these tracings



FIG. 7. DOG "G." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. Slow, continuous injection of diluted (1:5) cinnamon water between the arrows

with respect to peristaltic waves, the following experiments were performed: The balloon was filled with a fluid opaque to the x-ray (sodium bromide, 25 per cent solution) and introduced into the loop. The shadow of the balloon on the fluoroscope was watched before and during the action of a volatile oil, and the phenomena observed were recorded on the tracing made by the bellows recorder at the same time. In this way it was found that the marked increase in tone on the tracing occurred simultaneously with a broad constriction in the balloon that extended over the greater part of it; that, while this broad constriction was present there were narrower bands of constriction which corresponded with the rhythmic waves on the tracing, and further that some of these latter were stationary while others progressed over the entire length of the balloon. These moving constrictions usually passed from above downward, but some of them moved for a short distance in the opposite direction. This experiment showed that during a typical volatile oil effect, true peristaltic waves occur, especially during the increase in tone, but that it would be impossible from a study of the tracing alone to tell which of its waves represented stationary and which of them moving contractions.

To confirm and add to these observations made with the x-ray, a dog was anaesthetized with ether, the belly opened and belly walls pulled aside and upward so as to form a trough into which warm paraffin oil was poured until the coils of intestine were covered; the balloon, filled with water was then introduced into the ileum by making two small incisions in the gut wall; the contractions were recorded in the usual way. The contraction in the gut of this anaesthetized dog were very slight compared with those in the Thiry-Vella loop of an unanaesthetized dog. In this anaesthetized dog with the intestines exposed to direct inspection the effects of the volatile oils were of the same character as in the other experiments but they were slight in degree; the tracing showed increase in tone and in the amplitude of the rhythmic contractions. When the phenomena observed in the gut by inspection were recorded on the tracing, it was found that one could not distinguish on the tracing the waves caused by a

progressing contraction (peristalsis) from those produced by stationary rings. Both types of contraction rings could be seen in the gut wall over the balloon, particularly during the increase in tone that followed injection of volatile oil solutions into the gut, or intravenous injection of physostigmine sulphate and barium chloride, or after mechanical stimulation of the gut wall.

EFFECTS OF OTHER DRUGS ON THE ACTION OF VOLATILE OILS

Atropine. When atropine sulphate in doses of 1 to 3 mgm., is injected hypodermically into a dog with a Thiry-Vella loop, all the activities of the loop are decreased: general tone is lowered, tonus waves on the tracing are diminished or disappear and the amplitude of the rhythmic contractions is lessened. Doses of atropine of this order increased the pulse rate in these unanaesthetized dogs from 70-90 to 216-252 per minute. In such atropinized dogs the introduction of volatile oil solutions into the loop produced the same sort of changes in the tracing as before the atropine was injected, but the degree of effect was much reduced. The atropine reduced the usual tone increase that follows application of volatile oils more than it did the augmentation of the rhythmic contractions (fig. 8).

Cocaine. To obtain data on the relative importance of the sensory mechanism in the mucosa of the loop in its reaction to volatile oils the following procedure was carried out: the reaction of the loop to cinnamon water was first recorded, then 25 cc. of a 4 per cent solution of cocaine hydrochloride were introduced and allowed to remain for five minutes; it was then washed out. The application of cocaine stopped the tonus waves and diminished, for a time, the rhythmic contractions. These effects were not as pronounced as those from hypodermic injection of atropine. In the cocainized loop undiluted cinnamon water and diluted (1:10) spirit of cinnamon had no effect whatever on the tracing. Paralysis of the sensory mechanism in the mucosa of the loop abolishes the effect of solutions of volatile oils. Both dogs gave identical results (fig. 9).

Morphine. It seemed possible that during a marked increase in the muscular activities of the intestine, the volatile oils might produce effects that differed materially from those ordinarily obtained. To test this possibility, advantage was taken of the fact that in dogs small doses of morphine induce marked augmentation of intestinal tone and contractions. Thus, when 10 mgm. of morphine sulphate were injected hypodermically into a dog with Thiry-Vella loop, there was a general increase in the

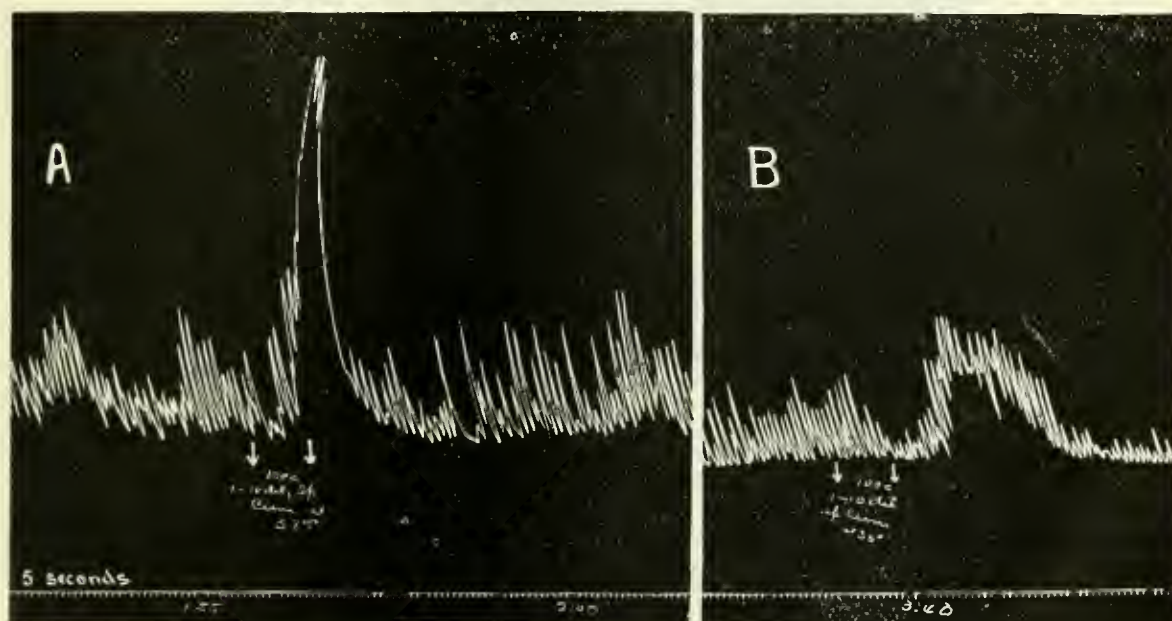


FIG. 8. DOG "L." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. A, shows the effect of injecting into the loop 10 cc. spirit of cinnamon diluted 1:10, before atropine. B, shows the effect of repeating the injection eighteen minutes after giving 2 mgm. atropine sulphate hypodermically.

tone of the loop, marked tonus waves occurred and the rhythmic contractions were augmented. This dosage of morphine did not cause active vomiting though evidences of nausea (salivation) were usually present. Introduction of volatile oil solutions into the loop during this increased activity from morphine gave essentially the same effects as before morphine was injected (fig. 10).

Most of the experiments on which this report is based were made with solutions of the oils of peppermint and cinnamon.

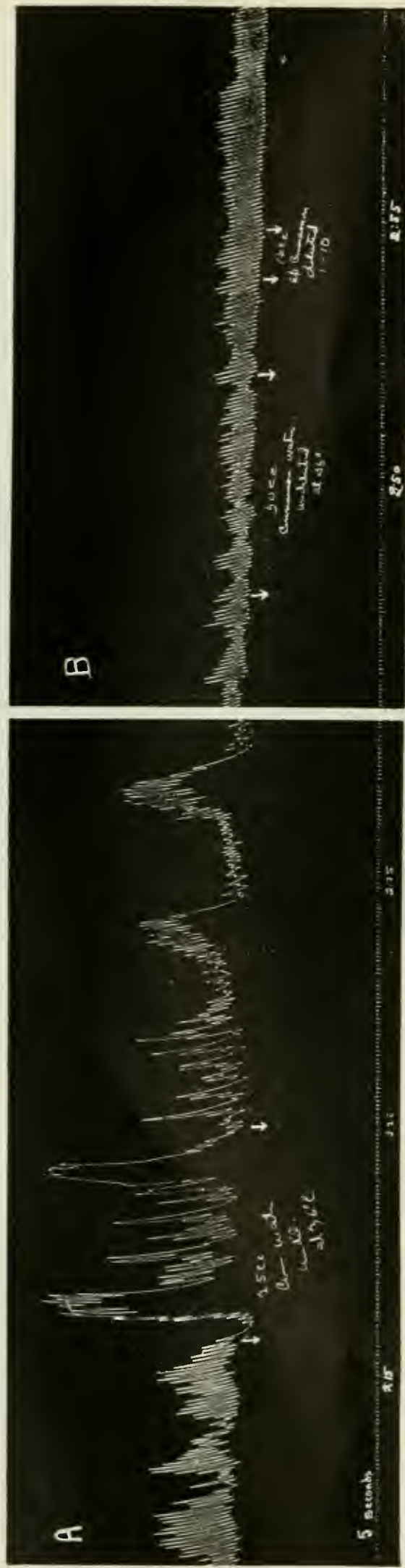


Fig. 9. Dog "G." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. A, shows the effect of introducing 25 cc. of cinnamon water into the loop before application of cocaine. B, shows the effect of 50 cc. cinnamon and later of 15 cc. spirit of cinnamon 1:10 dilution, introduced ten minutes after application of 4 per cent solution of cocaine hydrochlor. to the loop.

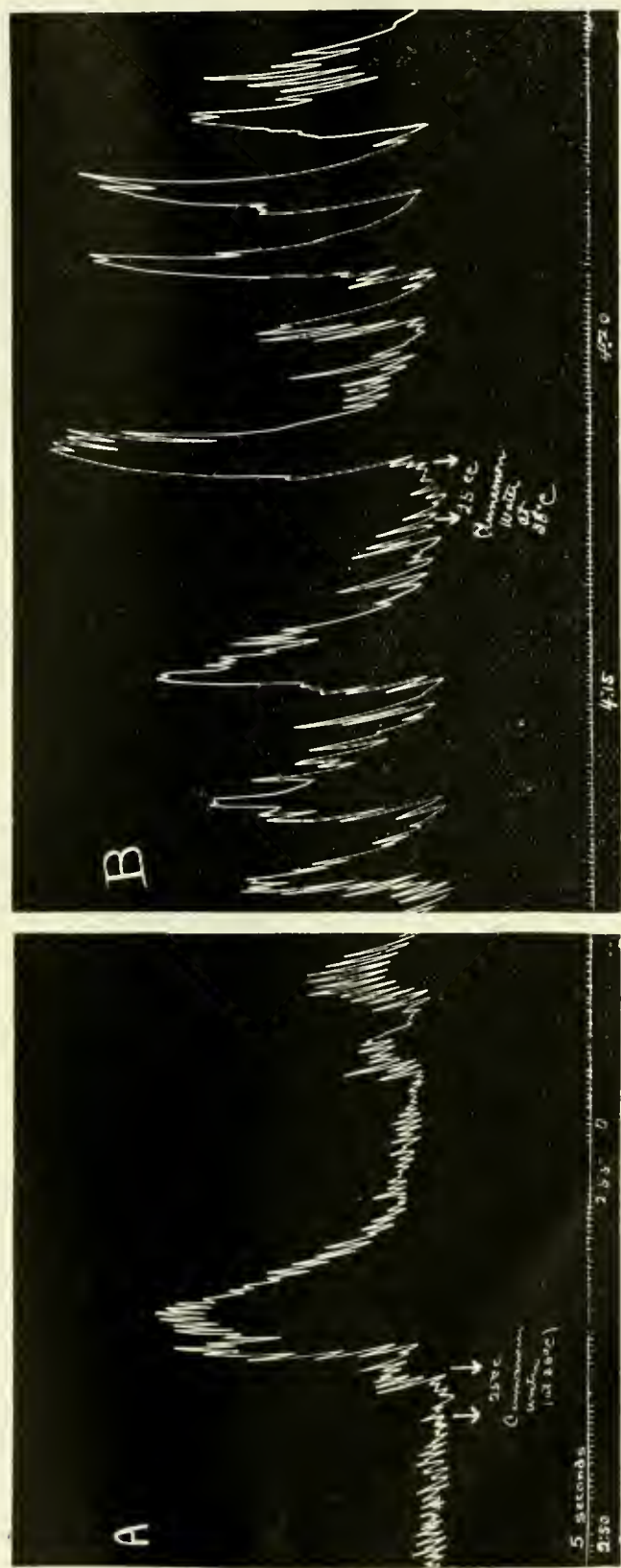


FIG. 10. Dog "L." BALLOON FILLED WITH WATER

Large bellows recorder. Slow drum. A, shows effect of introducing 25 cc. cinnamon water into the loop before morphine was injected. B, shows same, fifty minutes after hypodermic injection of 10 mgm. morphine sulphate.

Solutions of the following were also tested: the oils of cloves, anise, nutmeg, lavender, juniper, cardamon, spearmint, fennel, orange, caraway and the volatile oil of mustard as well as the stearoptenes, menthol and camphor. All of these volatile oils and stearoptenes produce the same kind of effects when placed in a Thiry-Vella loop, but there is considerable variation in the amount of effect which different ones call forth. While the experiments were not of a character that enables one to state these differences quantitatively, it is possible from the results obtained to arrange the oils roughly into two groups which merge more or less into each other: thus, the *stronger* oils produce a pronounced effect in dilute solution, the *weaker* ones show less effect and require stronger solutions to bring it about. In the former group would be included, the volatile oils of mustard, cinnamon, peppermint, cloves, nutmeg, spearmint, juniper and lavender as well as menthol and camphor; in the latter the oils of anise, cardamon, fennel, orange and caraway.

SUMMARY

1. Carminative volatile oils increase the muscular movements in the intestine when applied to the mucous membrane in dilute solution in unanaesthetized dogs.
2. This increased activity involves an augmentation of tone and of rhythmic contractions, and, at least during the increase in tone, progressing contraction rings (peristalsis) occur.
3. Occasionally the primary increase in muscular activity is followed by a decrease in tone and in amplitude of the rhythmic contractions.
4. These effects of the volatile oils are lessened but not abolished by atropine. They are abolished when the sensory endings of the mucosa are paralysed by cocaine.
5. When the muscular activities of the intestine are markedly increased by the injection of small doses of morphine in dogs, the same kind of effect is produced by the volatile oils as before the morphine was injected.

I wish to express my appreciation and thanks to Prof. H. K. Pancoast for his assistance and the use of his laboratory in carrying out the x-ray observations; and to Prof. A. N. Richards and Dr. C. F. Schmidt for their coöperation in various phases of the investigation.

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THE ACTION OF CAFFEINE, THEOBROMINE AND THEOPHYLLINE ON THE MAMMALIAN AND BATRACHIAN HEART

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I. LITERATURE

The action of caffeine on the circulation has been investigated by means of several kinds of experiments, on the intact animal, on the isolated mammalian heart, on surviving arteries, both systemic and coronary, and on the frog's heart.

The intact animal

Pilcher (17) has examined the action of caffeine on the heart. He found that, during the acute fall of blood pressure following intravenous injection of caffeine, if the dose was less than 10 mgm. per kilo the heart volume and amplitude remained unchanged, but if the dose was greater, the diastolic volume increased and the amplitude lessened. The permanent changes resulting from not more than 20 mgm. per kilo were a moderate rise of blood pressure and an increase in both heart rate and amplitude. With larger doses there resulted a progressive fall of blood pressure, an increase of rate and diastolic volume and a fall in amplitude.

Sollmann and Pilcher (23) state that the vagus center is stimulated and that caffeine in small doses may cause slowing of the heart from this cause but that the direct effect of large doses always outweighs this and causes an increase in rate. Further they have investigated the action on the blood pressure and on the peripheral vessels of increasing doses of caffeine. They found that on intravenous injection of small doses, there was a

momentary depression of the myocardium and a consequent fall of blood pressure. This was promptly succeeded by a rise of blood pressure due to stimulation of the myocardium. There is at the same time vasodilation but, the output of the heart being increased more than is necessary to compensate for the dilation of the peripheral vessels, the blood pressure rises. With larger doses there is a permanent fall of blood pressure owing to cardiac depression and vaso-motor paralysis.

In their paper is given an excellent summary of the previous literature.

There appear to be few or no references to the action of theobromine on the intact animal. With theocine, Hedinger (14) found only a slight increase in blood pressure in rabbits which had been made nephritic with compounds of uranium and chromium. There was at the same time a considerable increase in the kidney volume.

The isolated mammalian heart

Hedbom (13) using Langendorf's method found that caffeine increased both the rate and amplitude of the heart beat. Further the output from the coronary vessels was raised, sometimes to a large extent, with solutions ranging in strength from 1 in 20,000 upwards.

Loeb (15) using the same method obtained results similar to those of Hedbom as far as rate and amplitude were concerned but could only rarely obtain, and then, only in small degree, the increased coronary output with caffeine. Theobromine, however, gave large increases in rate, amplitude and coronary output.

Bock (5) using the heart-lung preparation described by himself found that caffeine and theobromine had an entirely similar effect on the heart rhythm. He considered that this was due to an action on the accelerating cardiac ganglia and not to a depressant action on the vagus nerve endings. Further he thought that the output of the heart per beat was diminished and that vaso-motor constriction of central origin accounted for any rise of blood pressure in the intact animal by more than neutralising the reduced cardiac output.

Plant (18) using the Knowlton-Starling heart-lung preparation found that the rate, amplitude and total output of the heart were increased by caffeine. There was also a rise of pressure in the tube leading to the artificial resistance. The output of the heart per beat was not altered and the relaxation of the heart was slightly reduced.

Beco and Plumier (4) using the Langendorf method perfused the hearts of dogs which had received a dose of chloral. They found that both theocine and agurine increased the rate and amplitude. They apparently did not record the coronary output.

Rabe (19) using the same method could find no appreciable change to be produced in the heart by caffeine in strengths ranging from 1 in 5000 to 1 in 40,000.

Surviving arteries

Cow (7) found that rings of renal and splenic arteries dilated in a solution of caffeine but that the carotid was unaffected.

Eppinger and Hess quoted by Rabe (loc. cit.) found that strips of coronary artery lengthened in a solution of caffeine.

Beco and Plumier (loc. cit.) found that, in a kidney excised and perfused with defibrinated blood, an addition of caffeine caused an increase in the outflow from the renal vein, clearly due to vaso-dilation.

Sollman and Pilcher (loc. cit.) showed that the vessels of the kidney and spleen when previously contracted with adrenaline were easily caused to dilate by perfusion with a weak solution of caffeine.

Meyer (16) using his method of recording the flow from a coronary vein found that an injection of caffeine intravenously caused an increase in the rate of flow. He considered this to be due to the increased systemic blood pressure.

Sakai and Saneyoshi (20) using a similar method found that caffeine and diuretine in small (sub-therapeutic) doses constricted the coronary arteries, in average therapeutic doses dilated them and in large toxic doses dilated them very largely.

Frog heart

Aubert (3) injected caffeine into the dorsal lymph sac. He found that large doses slowed and small doses accelerated the heart.

Dreser (10) found that the output per beat and absolute power of the heart were increased.

Other references cited by Sollman (22) as dealing with the frog's heart are unfortunately not available.

There seems to be a lack of definite evidence as to the action of all three drugs on the frog heart, more especially as regards theobromine and theophylline. A certain amount of conflict appears to exist between the reports of the various observers as to their action on the isolated mammalian heart, particularly as regards the coronary output.

II. METHODS

Caffeine was used as the free base as it is fairly easily soluble in water or in Locke's solution and the complications that might arise from the presence of citric acid are avoided.

For the experiments with theobromine on the frog's heart and, in the majority of cases, on the mammalian heart also, the free base was employed. It had at first been thought that diuretine, the double salt of theobromine with sodium salicylate, would have been more useful owing to its much greater solubility in water. Diuretine was found however to be readily precipitated by calcium salts and a saturated solution of diuretine in Locke's solution contained about one part in a thousand or one in two thousand of theobromine as diuretine contains about 50 per cent of theobromine. A saturated solution of theobromine in Locke's solution contains about one in fifteen hundred so that there is at least as much of the active principle in this as there is in a saturated solution of diuretine in the same medium. Agurine, the similar double compound of theobromine with sodium acetate, was not available.

Theophylline as the free base could not be obtained and recourse was had to theocine, the double salt with sodium acetate.

It contains about 70 per cent of theophylline and is not readily precipitated by calcium.

For the frog's heart a modification of Symes' (25) method was employed and for the mammalian, Gunn's (11). In the case of the frog the cardiac, and in that of the mammal the coronary, output was measured using Gunn's (12) syphon recorder.

In the following pages the concentrations of both theocine and diuretine are given in terms of theophylline and theobromine the active substances. Thus a 1.4 per cent solution of theocine, equivalent to 1 per cent of theophylline, was prepared and the requisite dilutions made from this. The solution in Locke's solution may become pink on keeping but apparently without change in its activity.

III. EXPERIMENTAL

a. Frog heart

Preliminary experiments showed that no appreciable results were to be obtained with these drugs in the concentrations used for the mammalian heart. In consequence 1 per cent solutions of caffeine and theophylline were used as maximum strength. Of course theobromine could only be employed at a strength of 1 in 1500 as a maximum.

A tracing (fig. 1), is shown obtained with theophylline in a concentration of 1 in 1500. This may be taken as a standard to which all the other tracings obtained may be referred.

The first effect to be observed is a simultaneous decrease of the excursions towards systole and diastole. With theophylline, this is invariably present with solutions of this or any greater strength. It may occur even with 1 in 5000. This immediate diminution of the excursion toward systole never appeared with any strength of caffeine or theobromine used but with caffeine the diminution towards diastole occurred within about the same limits of strength of solution as with theophylline. With lower strengths of caffeine the change was often later in appearing than with similar strengths of theophylline. No decrease of the relaxation in diastole was observed with any strength of theobromine.

After this immediate effect is seen a period when both contraction and relaxation were increased with a consequent increase of amplitude. In the tracing this phase lasts for six minutes with no appreciable diminution and it has been seen to last for much longer. With strong solutions, however, the primary decrease of diastole persists and is not replaced by an increase but may be greater than the change in systole and a fall in the amplitude occurs. This is the case pretty regularly with solutions of 1 in

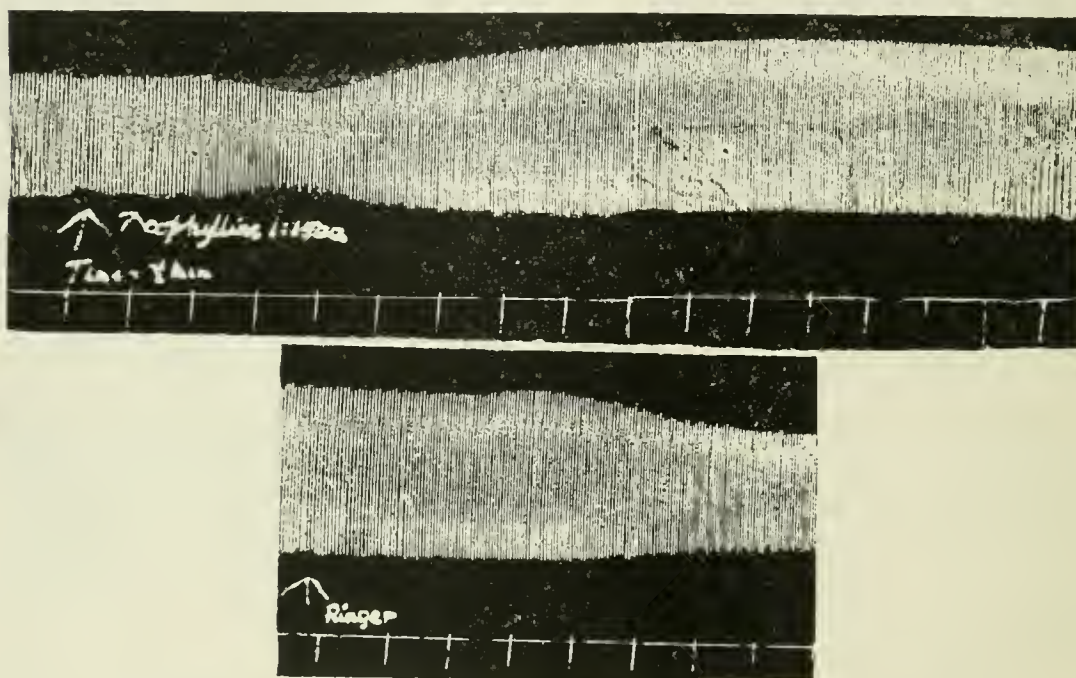


FIG. 1. ISOLATED FROG HEART

Theophylline 1:1500. Tracing shows (1) simultaneous diminution of systolic and augmentation of diastolic tone; (2) period of increased systolic and diminished diastolic tone with increased amplitude; (3) small increase of systolic tone on perfusing with Ringer's solution; (4) final recovery.

400 and stronger but is not often seen below this strength. With weak solutions the amplitude is increased from the commencement.

With caffeine the tracings obtained are very similar to these, amplitude being reduced with strengths from 1 in 100 to 1 in 400, being at first reduced and later increased between 1 in 500 and 1 in 1300 and being increased from the commencement below the latter strength.

Theobromine produces an increase of amplitude from the commencement, there being at no time a decreased relaxation.

On changing over the perfusion fluid to Ringer's solution there will be seen in the tracing a small increase of systole. It is only momentary in time but is remarkably constant in its appearance with all strengths of theophylline down to 1 in 2000. After perfusing with strong solutions this increase of systole is accompanied by a simultaneous lessening of relaxation, sufficient sometimes to cause a reduction in amplitude. The cause of this is obscure but may conceivably be analogous to that of Straub's phenomenon with muscarine (24). This was never seen to occur with either caffeine or theobromine.

In the tracing the cardiac frequency will be seen to be raised. With each of the three drugs in weak solution this was the case. With strong solutions of caffeine the heart rate is reduced from the commencement but with theophylline there is a brief period of acceleration before depression sets in. With both, in concentrations from 1 in 500 to 1 in 1500, there is at first a period of increased frequency which is often followed, in the case of caffeine, by slowing of the heart after twenty minutes' perfusion.

The changes in cardiac output generally followed directly the changes in the relaxation. When there was only a slight change in diastole the output was more influenced by changes in frequency than by those in the systole.

The lower limit of activity was found to be reached with each of these drugs at about the same degree of dilution, about 1 in 5000 to 1 in 10,000. After perfusion with solutions of any of these drugs in concentration not greater than 1 in 1500 recovery with Ringer's solution is complete in each case. With stronger solutions it cannot be depended on and after solutions stronger than 1 in 400 is rarely or never seen.

Occasionally as stated by Symes a heart was found to beat quite regularly but at a slow rate. A tracing of one of these perfused with caffeine 1 in 300 is shown (fig. 2). The great diminution of relaxation which occurs with solutions of this strength can be well seen.

Caffeine was the only one of the three drugs which was found to stop the frog's heart in systole and then only when perfused in a concentration of 1 in 100. This is perhaps curious as its effect in producing rigor in voluntary muscle can be seen in a dilution of 1 in 2000.

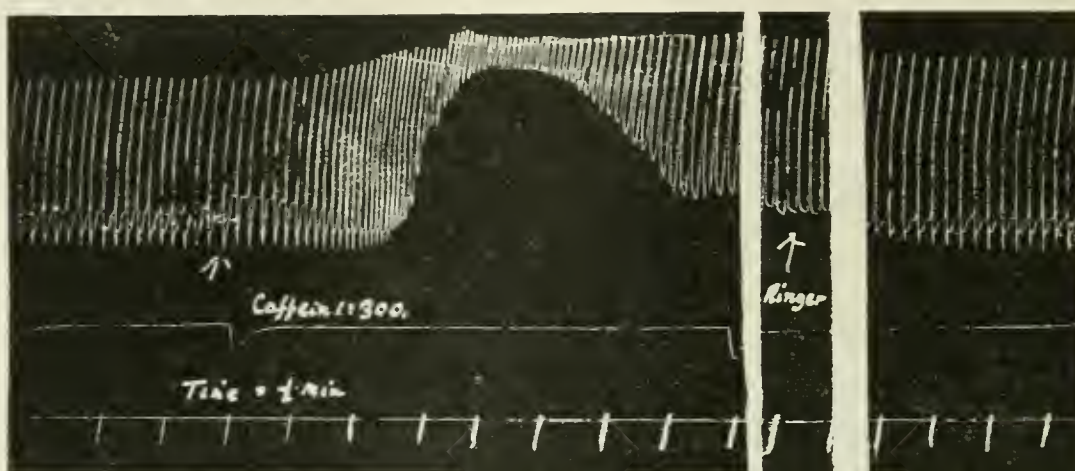


FIG. 2. ISOLATED FROG HEART

Slow heart. Perfused with caffeine 1: 300. Tracing shows (1) increased frequency; (2) small rise of systolic and very large rise of diastolic tone which later falls off; (3) recovery with Ringer's solution.

b. Mammalian heart

For these experiments young rabbits from nine to sixteen weeks old were used. One tracing each, obtained with caffeine, theobromine and diuretine in a concentration of 1 in 5000 of active substance and with theophylline in 1 in 7500, is shown (figs. 3 to 6). Diurétine was not used for many experiments and, as its effects appear to be practically identical with those produced by theobromine, it may be dismissed from further consideration with the note that, possibly owing to its 50 per cent content of sodium salicylate, it appeared to be somewhat more toxic than theobromine.

The general effects of these drugs can be made out from the tracings. In all there is a rise of systole, in cardiac frequency and in coronary output. Sometimes there may be, especially with the stronger solutions, a diminished relaxation either immedi-

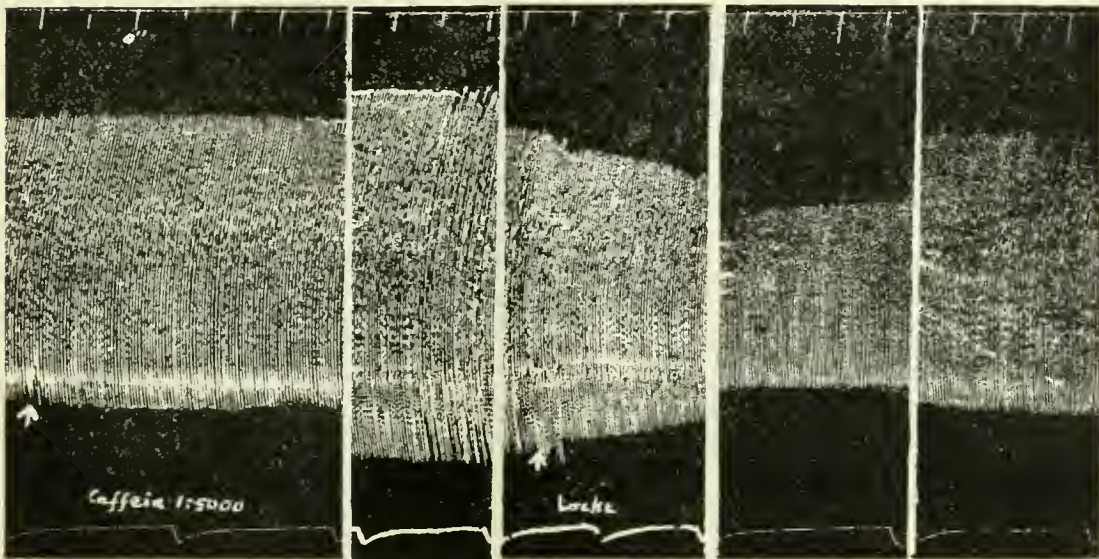


FIG. 3. ISOLATED RABBIT'S HEART

Perfused with caffeine 1: 5000. Tracing shows 22 per cent increase of frequency 28 per cent increase of amplitude with a late fall of systolic tone and 50 per cent increase of coronary output. Recovery with Locke's solution.

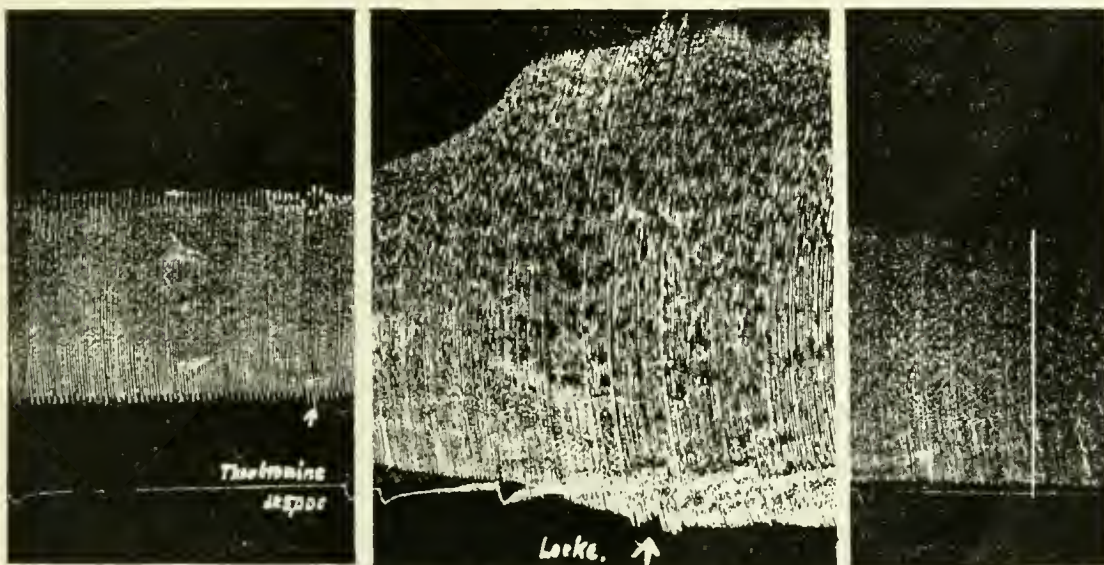


FIG. 4. ISOLATED RABBIT'S HEART PERFUSED WITH THEOBROMINE 1: 5000

Tracing shows 54 per cent increase in frequency, 145 per cent increase in amplitude and, though rather difficult to make out, 170 per cent increase of coronary flow. There is some permanent lowering of both systolic and diastolic tone.

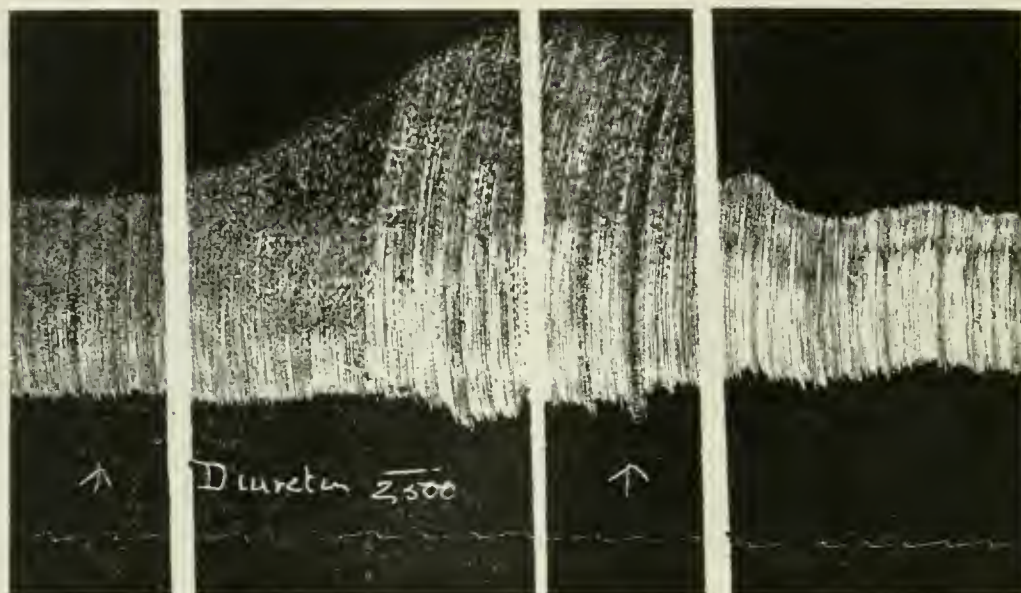


FIG. 5. ISOLATED RABBIT'S HEART PERFUSED WITH DIURETINE 1:2500, I.E.,
THEOBROMINE 1:5000

Tracing shows 60 per cent increase of frequency, 100 per cent increase of amplitude and 30 per cent increase of coronary output. The flow was rapid to begin with, the vessels being nearly fully relaxed and in consequence the increase of coronary flow is not very marked.

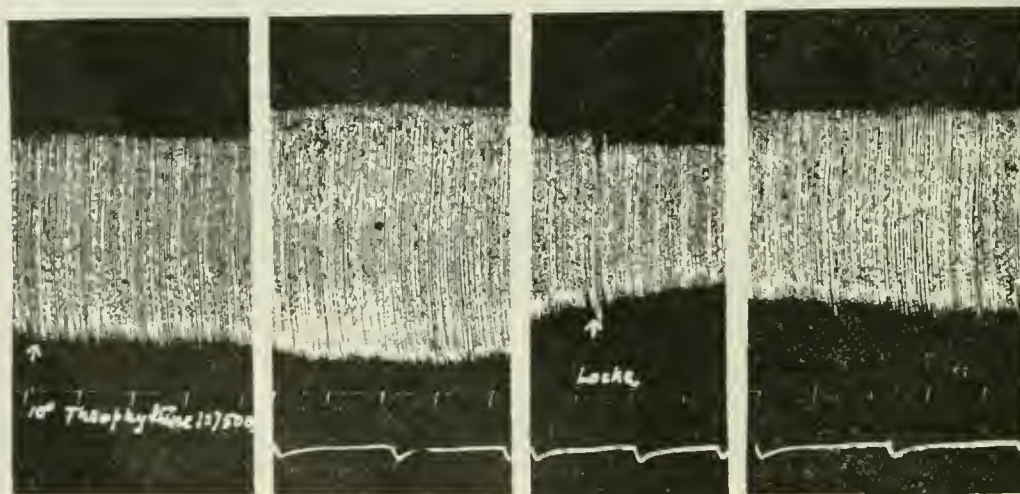


FIG. 6. ISOLATED RABBIT'S HEART PERFUSED WITH THEOCINE 1:5000, I.E.,
THEOPHYLLINE 1:7500

Frequency increased 22 per cent, amplitude 30 per cent, coronary flow 50 per cent. Recovery with Locke's solution complete.

ately or, as in the theocine tracing, after an interval of time. With the weaker solutions it was generally observed that there was little or no change in diastole and that such change as might appear was in the direction of increased relaxation.

The experiments were carried out with all the drugs in solutions ranging from 1 in 2000 to 1 in 40,000. It was found that the lowest effective strength to cause an increase of the coronary flow was, of caffeine 1 in 10,000 to 20,000, of theobromine 1 in 40,000 and of theophylline 1 in 30,000 to 40,000. Alterations in cardiac rhythm and amplitude could still be obtained with solutions of about two thirds of this strength for each drug. It will be seen therefore that the coronary vessels are less sensitive to these drugs than is the cardiac musculature.

The time relations of the onset and disappearance of the changes in cardiac rhythm and amplitude and of coronary flow are worthy of consideration. Generally speaking, the increase of frequency and of augmentation of the beat are synchronous or nearly so, both in their onset and passing off. The increase of coronary output shows no constant time relation to that of either of the others. Perhaps on the whole it is later in coming on and earlier in disappearing.

In table 1 are collected the average percentage alterations obtained with each drug. Reference to it will show that caffeine in a concentration of 1 in 2000 has an effect on coronary output and on frequency which is greater than that produced by either of the others in the same concentration but less than by them on amplitude. Its action on the coronary output falls off rapidly with increasing dilution while in the case of theophylline there is a rise to a maximum at 1 in 7500 and in that of theobromine to a maximum at 1 in 5000. In consequence at these concentrations and with further dilution so long as any effect is obtained theophylline and theobromine have a greater effect on the coronary flow than has caffeine.

As far as the cardiac stimulant action is concerned there is perhaps not much to choose among the three except that theophylline and theobromine still can produce some effect in a dilution at which caffeine produces no alteration.

IV. DISCUSSION

The object of this work has been (1) to obtain some clear idea of the action of these drugs on the frog's heart, both absolutely and comparatively (2) to supplement the work of earlier investigators and to examine and compare their action on the mammalian heart with especial reference to the coronary flow.

The frequency and amplitude of the heart beat

It has been shown that in the excised heart of both frog and mammal these drugs accelerate and augment the heart beat when perfused in suitable dilutions. This might be due to either a stimulation of the accelerator nerve endings or a direct action on the cardiac musculature. Against the first view we have the experiments of Cushny and Van Naten (8) and of Dixon (9). The former found that caffeine when injected into a dog produced a change in the cardio-myographic tracing quite unlike that elicited by stimulating the accelerator nerve. The latter showed that after an injection of apocodeine to paralyse the sympathetic nerve endings injections of caffeine produced their normal effect. We must then conclude that cardiac, like skeletal, muscle is stimulated directly by these drugs.

The coronary output

It was found that all three drugs when perfused through the rabbit's heart increased the flow through the coronary vessels. Pressure remaining constant in the perfusion apparatus there can be no question of increased aortic blood pressure due to an increased action of the heart causing this rise in the coronary flow as might be the case in the intact animal (cf. Meyer (16)). The increased flow might be due to the increase in rate and amplitude or to a direct vaso-dilator action on the coronary vessels.

The amplitude being a measure of the power of the heart's contraction, it is possible that a more complete emptying of the coronary vessels will accompany a more powerful contraction of the heart and so an increase of coronary flow result. Such an increase is not likely to be very great.

The effects of changes in the frequency of the heart on the coronary circulation are not so easy to determine. The greater part of the flow must take place during the period of diastole and with an increased rate of beat the period of diastole must be reduced to a proportionally greater degree than that of systole. This would point to a probable diminution of coronary flow. On the other hand each contraction of the heart must force on the liquid contained in the coronary vessels to the right auricle and with an increased number of such contractions there would be an increase of the coronary flow.

If the coronary output were dependent on the rate and power of the heart beat it would be expected that changes in the flow would be synchronous with the changes in the rate and amplitude or would at least bear some constant time relation to them. This was not found to be the case. Tracings have been obtained in which the coronary flow was the first to show any alteration and the last to return to the normal, others show the exact opposite, the coronary flow being the last to show an increase and the first to return to the normal. Again some tracings have been found to show the increase in coronary flow before the increase in rate and the disappearance of the increase in output before that of the rate.

If there were a diminution of relaxation of the heart muscle it would be probable that the coronary vessels buried in that muscle would be somewhat constricted by the imperfect relaxation and that the coronary flow would in consequence be diminished unless there were some active cause operating in the opposite direction. In figure 7 is shown a tracing obtained with caffeine, 1 in 10,000. In this tracing it will be seen that although there is no increase in frequency and there is an actual diminution of the amplitude (due about equally to diminution of contraction and of relaxation) there is a considerable increase of coronary flow amounting to about 20 per cent. In figure 8 obtained with theobromine 1 in 20,000, there is no increase of amplitude, in fact a slight fall of about 10 per cent, accompanied by an increase of 10 per cent in rate. The coronary output on the other hand shows an increase of 20 per cent. These two tracings make it

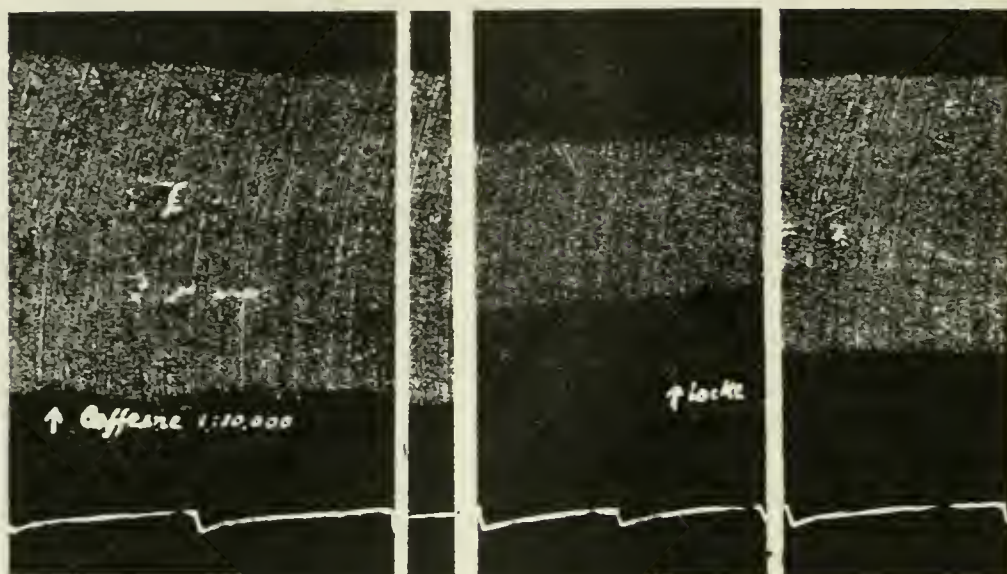


FIG. 7. ISOLATED RABBIT'S HEART

Caffeine 1:10,000. Late diminution of excursions towards diastole and systole with contemporary increase of coronary flow. Recovery with Locke's solution. Rate increased nil. Amplitude at first increased 5 per cent, later decreased 60 per cent. Coronary output rose 20 per cent.

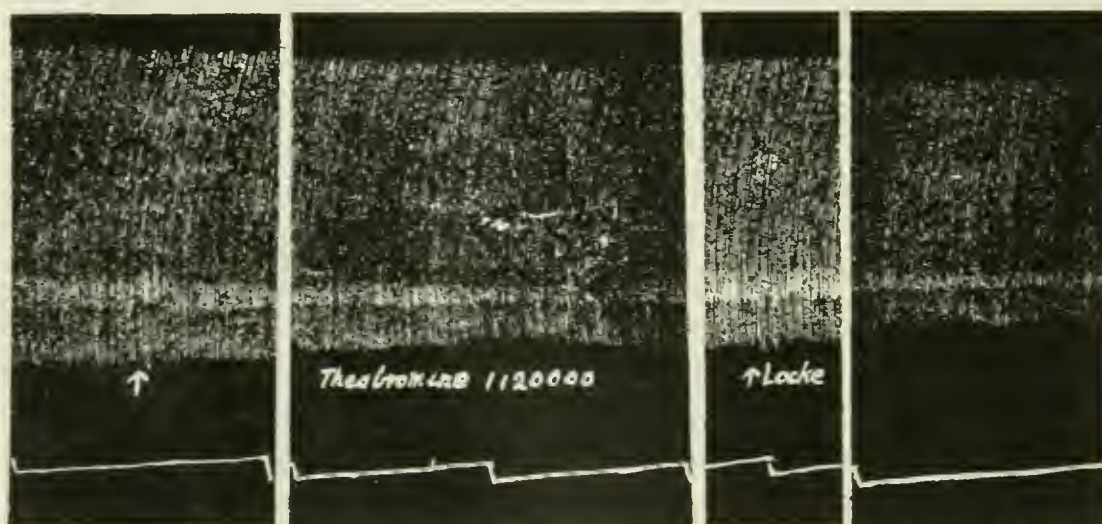


FIG. 8. ISOLATED RABBIT'S HEART

Theobromine 1:20,000. Rate increased 11 per cent. Amplitude, at first unchanged, later fell 10 per cent. Coronary output increased 20 per cent.

difficult to understand how changes in rate and amplitude can be entirely responsible for changes in coronary output.

If reference be made to table 1, in which are collected the average percentage changes obtained with these drugs in different dilutions, it will be seen that with caffeine, 1 in 20,000, the rate was increased by 16 per cent and the amplitude by 25 per cent while there was no change in the coronary flow. With theobromine, 1 in 10,000 there was an increase of 18 per cent in rate and of 23 per cent in amplitude while the coronary flow was increased by 36 per cent. In these two cases representing the average changes obtained with the drugs in these concentrations we find increases in rate and amplitude which are the same within the error of experiment and which should give the same changes in coronary flow if this is dependent on the changes in rate and amplitude. We find on the other hand a marked difference to exist.

On this evidence we are entitled to assume that the cause of the increased coronary flow is a vaso-dilation produced by these drugs probably by a direct action on the vessel wall. This is in keeping with the results obtained from other sources. Eppinger and Hess (quoted by Rabe, (19)) found that strips of coronary artery lengthened in a solution of caffeine. Cow (7) found that there was a similar action on rings of renal and splenic arteries. Sollman and Pilcher (13) showed that the vessels of the kidney and spleen previously contracted by adrenaline were caused to dilate by perfusion with caffeine.

If, as has been thought by some, the cause of Angina Pectoris or of Cardiac Asthma is to be found in a condition of spasm of the coronary arteries it would be reasonable to attempt to combat these diseases by the use of these drugs. It is, at any rate, interesting to note that theobromine has been recommended for such use by physicians. Apparently Askanazy (2) was the first to employ theobromine in these cases and he was soon after followed by Breuer (6). Both seem to have met with a good deal of success.

If the blood volume of an average man be reckoned to be about 5000 cc. a dose of 5 grains, if it was all in the blood at one time, would represent a strength of about 1 in 15,000. Reference to

Table 1 will show that at this concentration theophylline and theobromine are still capable of producing an increase in the coronary flow while caffeine is on the verge of losing its power in this direction. It would seem then that of the drugs, theobromine and theophylline are to be preferred to caffeine for this purpose. Theophylline has been thought to have caused acute cases of poisoning in man and in experimental animals (Allard 1). Schmiedeberg who quotes (21) a long list of fatalities did not think that the drug was to be blamed for the deaths. However this may be, theobromine does not suffer from this disadvantage

TABLE 1

To show effects of caffeine, theophylline and theobromine on rabbit's heart; average percentage increase

STRENGTH	CAFFEINE			THEOPHYLLINE			THEOBROMINE		
	Rate	Ampli- tude	Coro- nary output	Rate	Ampli- tude	Coro- nary output	Rate	Ampli- tude	Coro- nary output
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1:2000	61	51	120	35	140	20	30	80	50
1:3000				31	100	23			
1:4000							19	80	40
1:5000	28	27	50	53	40	33	44	100	180
1:6000							23	30	14 ?
1:7500				20	37	50			
1:10,000	22	33	20				18	23	36
1:15,000				22	35	45			
1:20,000	16	25	Nil	10	23	22	17	14	8
1:30,000				15	33	30			
1:40,000	Nil	Nil	Nil	16		Nil	4	8	.6

and is nearly as active in its effects on the coronary vessels as theophylline. It would seem that theobromine, of these drugs is the one to be preferred in cases where a dilator action on the coronary vessels would be of service.

As a general cardiac stimulant it would again be better to exhibit theobromine rather than caffeine owing to the unpleasant side actions on the central nervous system which are so readily produced by the latter when administered in full doses (cf. Taylor (26)). Theobromine can be given in doses about four times as great as caffeine without causing any marked stimulation of the nervous system.

V. CONCLUSIONS

1. The action of caffeine, theobromine and theophylline in the frog's heart has been examined and compared.

2. The action of these drugs on the mammalian heart has been investigated with especial reference to the coronary circulation. At the same time the work of previous experimenters has been collated and confirmed as far as the effects on rate and amplitude are concerned.

3. It is claimed that all three have an active vaso-dilator action on the coronary vessels, probably muscular in origin, caffeine being the weakest and theobromine the strongest.

4. Experimental evidence is thus provided for the use of theobromine in those conditions such as angina pectoris in which coronary vaso-dilation may be of service.

Finally the author wishes to express his most grateful appreciation of the kindness of Professor J. A. Gunn to whom he is indebted for assistance in every way, for advice and, not least for most kindly criticism.

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COMPARATIVE STUDIES ON THE PHYSIOLOGICAL VALUE AND TOXICITY OF COTTON SEED AND SOME OF ITS PRODUCTS¹

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INTRODUCTION

It is a well known fact that certain animals die when fed on cotton seed products. The deleterious effect of such foods,

¹ The data in this paper were taken from the dissertation submitted by Icie G. Macy for the degree of Doctor of Philosophy, Yale University, 1920. Part of the expenses of this investigation was defrayed by a grant from the Russell H. Chittenden Research Fund for Physiological Chemistry.

resulting in the so-called "cotton seed meal injury," has been attributed to cholin, betain or their decomposition products, high protein content, careless feeding, lack of cleanliness, obstruction of the intestines by fibers, parasitic organisms, decomposition products of meal by microorganisms, "toxins," oil content, toxic phosphorus compounds, deficient diet, and recently to gossypol, an isolated phenolic compound.

A study of cotton seed meal injury is difficult owing to the variability of effects on animals consuming similar amounts of the same cotton seed food, the tardiness in appearance of abnormal symptoms in some cases, and the lack of uniform results; even animals of the same species respond at different times with unlike symptoms although they consume like quantities of the same food. Other difficulties are encountered. The degree of toxicity of cotton seed seems to depend upon the variety of seed and upon the climate and soil in which they are grown. And again, the *meal*, made from the kernels, is greatly altered by the treatment in the process of manufacture. There is uncertainty as to the degree of the responsibility of gossypol for the toxicity, as results of investigators differ. All such factors lend difficulty to the study of the effects of cotton seed foods and render the present status of the problem uncertain.

Comparatively little is known concerning the chemical composition and the fate of cotton seed products in the alimentary tract of animals. Realizing the wide spread use of cotton seed meal and the lack of definite knowledge concerning the causes and effects of "cotton seed meal injury," an attempt has been made in this investigation to ascertain whether death was due to inanition, a deficient diet, or to a toxic substance.

PLAN AND METHODS OF THE PRESENT INVESTIGATION

Plan of feeding experiments with rabbits, guinea-pigs, and pigeons

In the present experiments rabbits, pigeons, and guinea-pigs have received the cotton seed products moistened with molasses, to render them palatable to animals, and reënforced with liberal quantities of cabbage which supplied roughage and other desir-

able dietary factors; mice have received the same cotton seed products supplemented with butter fat and inorganic salts. There is reason to suppose that both types of mixture furnish the known essentials of a complete diet. In general, the animals were kept under favorable hygienic conditions, were given fresh water daily, and were weighed at frequent intervals depending upon the duration of the experiment.

As a control, animals were fed upon a corn meal mixture,² with molasses and cabbage, upon which they maintained good condition for long periods of time; control mice were fed upon a supplemented cotton seed meal I ration.³ A definite quantity of freshly mixed food was given the individual animals and throughout the day they ate ad libitum; the following morning the residual material was weighed and the food intake ascertained by difference.

Withers and Carruth (1915 b) have called attention to the fact that rabbits and guinea-pigs eat neither cotton seed meal nor cotton seed kernels readily. Following their method of feeding, but elaborating and extending the work to pigeons and albino mice, the present investigators used a cheap grade of molasses,⁴ for moistening and also for rendering the various rations more palatable. Inasmuch as the rabbits, guinea-pigs, pigeons, and in some cases, mice received comparable food mixtures weights are tabulated for the food intake as the dry food plus molasses (5:1), thus making it possible to calculate the quantity of meal if so desired.

² Composition of corn meal mixture:

	<i>parts</i>
Yellow corn meal.....	1
"Gold Medal Flour".....	1
"Quaker Rolled Oats".....	1

³ The composition of the ration was as follows:

	<i>per cent</i>
Cotton seed meal sample 1.....	50
Butter fat.....	5
Mineral salt mixture.....	4
Lard.....	41

Cotton seed sample I in a supplemented ration had not been noticeably toxic to mice.

⁴ "Cherry Grove, pure cane, dark red molasses" was used in all experiments.

Difficulty and inconvenience were caused frequently by caged rabbits scattering the food with their feet, thus rendering of questionable accuracy, the computation of the daily intake, an undertaking which at best is none too precise. To obviate this difficulty a special feeding device in the form of a modified galvanized-iron chick cup, which can be hung in the wire of the cage,



was designed. A removable cover, of the same material, cut so as to make a rim about $\frac{1}{2}$ inch wide around the crest of the cup, the inner edge being turned down about $\frac{1}{8}$ inch, was made to fasten securely on the cup. (The details are illustrated in the picture.) The advantages of the rabbit cup are obvious and the writer found that unless such a mechanism was used, it was practically impossible in most cases, to ascertain the food intake, for these animals.

In general, the guinea-pigs and mice caused less trouble by scattering their food. In the case of the former, it was only necessary to fasten the feeding cup securely to the cage, and in the case of the latter to use a receptacle of such bulk that it could not be overturned.

The pigeons fall under a different régime of feeding as they tend to scatter all food materials freely; so that even the intake of finely chopped cabbage could not be estimated. Therefore the food mixture was moistened with enough water to make it stick together and made into pellets of such a size as could conveniently be forced through the open beak of the bird without the loss of materials. In this manner, 10 grams of dry food were fed daily, 5 grams in the morning and the rest in the afternoon. The pigeon was held quiet by a towel wrapped tightly about its body during this procedure. A glass rod was of value in forcing the food into the crop; but it was more satisfactory to pour a few drops of water into the open beak after the pellet had been forced in. The bird then swallowed naturally and the bolus of meal slipped down more normally. The objection may very properly be made that this method of feeding might have an unfavorable influence upon the health of the bird. Nevertheless, control pigeons were fed in the same manner for a period of five months without any detrimental effects.

Control feeding experiments with rabbits, guinea-pigs, and pigeons

Inasmuch as the diets of cotton seed products that we proposed to feed were of a single food material which might prove to be monotonous, it was desired to see how much of an adequate ration rabbits, guinea-pigs, pigeons, and mice would eat under similar conditions, and to ascertain whether environment, methods of feeding, or long duration of experiments, would have any detrimental effects upon control animals.

Two diets were selected for rabbits: (1) soy bean flour⁵ chosen

⁵ Analysis of soy bean flour (made by Cerco Company, Tappan, N. Y.):

	<i>per cent</i>
Protein.....	(about) 44
Fat.....	(about) 20
Cane sugar.....	(about) 10

because the percentage of protein is very high; it is made from an oil cake, similar in composition to cotton seed meal, and in its preparation passes through similar commercial processes; (2) the corn meal mixture. These rations were moistened with molasses (5:1) and fed to rabbits in precisely the same manner as were all cotton seed products, and supplemented daily with

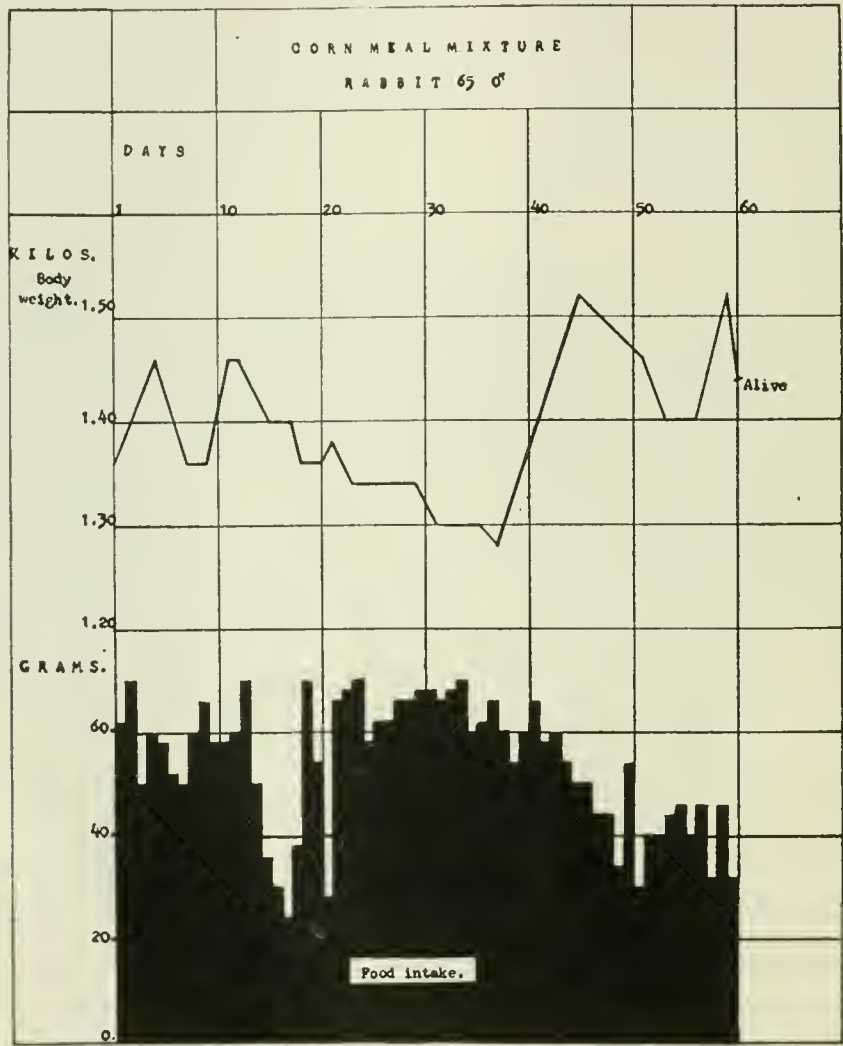


CHART 1

50 grams of cabbage. This amount of cabbage furnishes sufficient roughage and a liberal quantity of water-soluble B and antiscorbutic vitamins. Withers and Brewster (1913) observed that rabbits lived six to twenty-two days when fed cotton seed meal at the rate of 1 per cent of initial body weight. In view of this fact, two rabbits were fed for fifteen days on each of the

above mentioned diets. Both diets were adequate to approximately the same degree, but since the corn meal mixture was much cheaper it used as the standard control diet for all rabbits, guinea-pigs, and pigeons. Chart 1 is a typical illustration of the average daily food intake, and the maintenance of body weight for a rabbit.

After the selection of the corn meal mixture as the control diet, experiments were conducted on rabbits, guinea-pigs, and pigeons, over a long period of time. With only one or two exceptions, none of these animals lived longer than sixty days on any one of the cotton seed products employed, therefore this period of time was selected for a series of long time control experiments. All animals maintained approximately the same body weight throughout the experiment and at the conclusion were in good condition.

That molasses has no injurious effects when fed in a diet to such animals is also exemplified in this investigation. On the whole the control feeds were conducive to health, with no unpleasant symptoms appearing throughout the long time experiments. A few results are given in table 1.

TABLE 1

Animals fed on corn meal mixture

ANIMAL	DURATION OF EXPERI- MENT	AVERAGE DAILY FOOD INTAKE	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	REMARKS
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	
Rabbit 64 ♂	60	50	1720	1560	Alive
Rabbit 65 ♂	60	47	1360	1440	Alive
Guinea-pig 8 ♀	60	13	225	342	Alive
Pigeon 6 ♂	60	10	280	250	Alive

Control feeding experiments with mice

In view of the fact that Osborne and Mendel (1917) found that "satisfactory growth can be made by rats when either cotton seed globulin, or total cotton seed protein precipitated from alkali extracts of cotton seed meal, is employed without significant amounts of other protein in the mixture" and Richardson and

Green (1916) state that for rats "cotton seed meal does not contain sufficient mineral for growth, is not actively toxic, contains efficient proteins, and perhaps fat-soluble, growth promoting substances, similar to those of butter fat, but in less adequate quantities," a diet was prepared in which butter fat furnished the fat-soluble vitamine, Röhmann's salt mixture⁶ the necessary inorganic salts, and cotton seed meal I furnished the protein and other dietary essentials. Six mice, fed six months on this ration,⁷ increased in weight and maintained good appearance during which time one female successfully raised two litters of young, 2 and 5 respectively. Since this diet had proved to be satisfactory, it was adapted as the standard control diet for mice.

FEEDING OF COTTON SEED KERNELS⁸

Experiments with rabbits, guinea-pigs, and pigeons

The study of the toxicity of cotton seed is rendered more difficult by the variability in the effects on animals consuming similar quantities under apparently similar conditions. The effects are not sufficiently significant or uniform to give much exactness to experimental study of the question.

Unginned cotton seeds, together with the adhering cotton, were passed through a coarse meat grinder with the production

⁶ A synthetic salt mixture (Allgemeine Medizinische Central—Zeitung No. 9, 1908) was used. It consists of the following:

	<i>grams</i>
Calcium phosphate.....	10
Potassium acid phosphate.....	37
Sodium chloride.....	20
Sodium citrate.....	15
Magnesium citrate.....	8
Calcium lactate.....	8
Ferric citrate.....	2

⁷ Composition of control cotton seed meal ration:

	<i>grams</i>
Cotton seed meal I.....	50
Butter fat.....	5
Röhmann's salt mixture.....	4
Lard.....	41

⁸ These seeds were kindly furnished by Dr. O. F. Cook of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

of only a very slight amount of heat. The crushed mass was sifted through a coarse sieve, the kernels picked out and ground finely in a mortar, the resultant product being a meal which contained the whole kernel with very few hulls and little lint. This mash, containing all the oil and original constituents of the kernel, was mixed with molasses, as has previously been described, and fed to rabbits, guinea-pigs, pigeons, and albino mice; all food weights are given in *terms of molasses plus meal* (1:5). While some of the animals relished the material fed for the first few days, it was refused in part by some and wholly by others.

TABLE 2
Animals on "Lone Star" cotton seed kernels

ANIMAL	DURATION OF LIFE	FOOD INTAKE	ORIGINAL WEIGHT	FINAL WEIGHT	LOSS	
Rabbits						
	<i>days</i>	<i>grams</i>	<i>kilos</i>	<i>kilos</i>	<i>kilos</i>	<i>per cent</i>
31 ♂	5	17	1.46	1.18	0.28	19
32 ♂	12	38	1.70	1.32	0.38	23
33 ♂	32	336	3.26	2.02	1.24	38
35 ♂	26	96	2.50	1.82	0.68	27
Pigeons						
			<i>grams</i>	<i>grams</i>	<i>grams</i>	
S506 ♀	20	435?	263	160	103	39
11 ♂	11	110?	307	223	84	27
12 ♂	12	120?	265	170	95	35
Guinea-pig						
3 ♀	5	30	293	252	41	14

Rabbits ravenously consumed 50 grams of cabbage daily, even long after they had sickened on the cotton seeds; in most instances it was eaten until death, though with greater effort during the last day or so. On the contrary, the guinea-pig ate cabbage until the very end of life. In the cases of the rabbits and guinea-pig it was possible to determine the exact intake of cotton seed kernels as these animals do not vomit; the experiments with pigeons, even though the latter were forced-fed definite quantities daily in the manner hitherto described, were less accurate inasmuch as these birds ejected part of the food material. Cotton

seed kernels were very unpalatable to them; hence it was impossible to ascertain the exact amount of the unejected food intake. From table 2, it is evident that cotton seed kernels are not relished as a food and that all animals decline rapidly when fed exclusively on them. Apparently the added cabbage and molasses had no appreciable influence in stimulating the appetite for

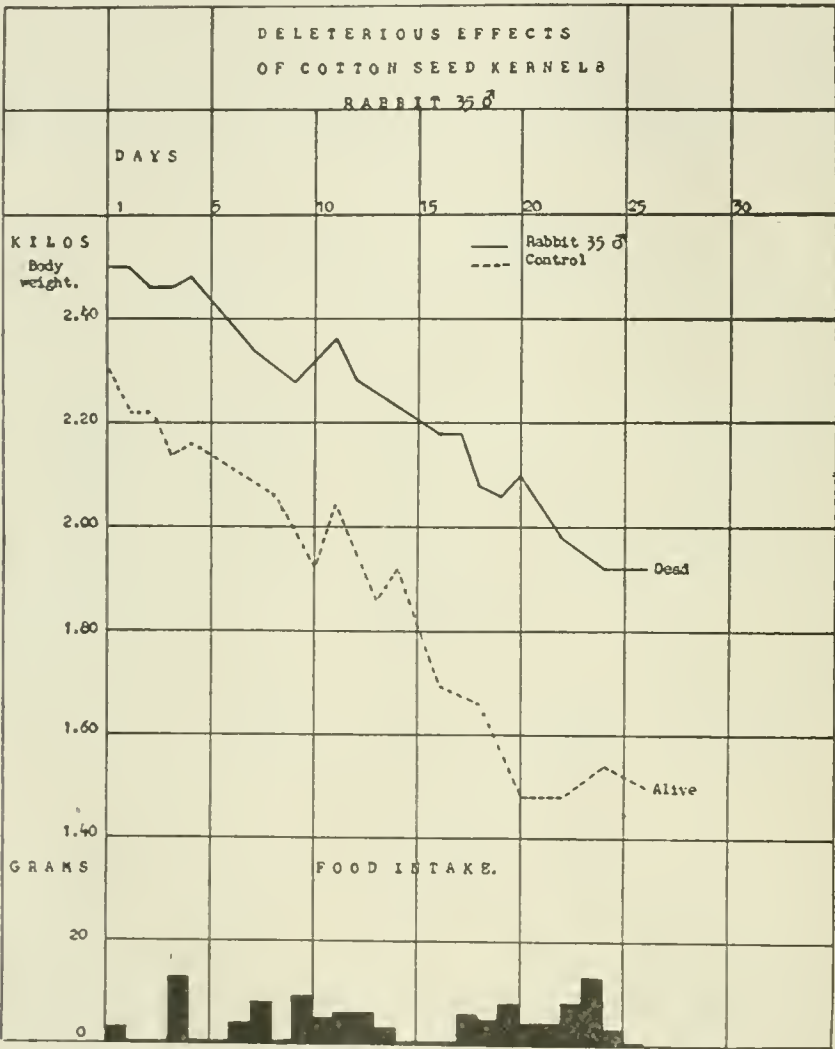


CHART 2

such a diet or in warding off its deleterious effects. Among control animals living under the same environment, receiving the same quantity of a diet which has been demonstrated to be adequate, fed in the same manner, no fatalities have resulted. Chart 2 is illustrative. Hence death was not due to starvation, though there was always emaciation, but to the harmful effect of the cotton seed kernels.

Experiments with mice

As will be observed in table 3 mice ate sparingly of all cotton seed *kernel* foods.⁹ Death might be due to either of two causes,

TABLE 3
Mice on cotton seed kernel food

MOUSE	DURATION OF LIFE	FOOD INTAKE	ORIGINAL WEIGHT	FINAL WEIGHT	LOSS	
Young mice						
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>
10 ♂	4	1.3	18.6	14.3	4.3	23
14 ♂	6	3.4	18.2	14.0	4.2	23
15 ♀	6	3.9	19.4	15.4	4.0	20
Large mice						
7 ♀	5	1.6	20.0	15.2	4.8	24
8 ♂	5	2.4	21.7	16.9	4.8	22
9 ♂	5	0.8	21.8	16.3	5.5	25
13 ♂	6	4.8	22.2	14.3	7.9	35

TABLE 4
Mice without food

MOUSE	DURATION OF LIFE	ORIGINAL WEIGHT	FINAL WEIGHT	LOSS	
Young mice					
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>
16 ♂	2	14.7	11.6	3.1	21
17 ♂	2	14.2	10.8	3.4	23
21 ♀	3	17.4	11.0	6.4	36
23 ♂	3	18.7	11.8	6.9	36
Large mice					
22 ♂	4	21.1	12.8	8.3	39
36 ♀	3	25.0	16.5	8.5	34
37 ♂	4	20.5	17.2	3.3	11

	<i>per cent</i>
⁹ "Lone Star" cotton seed kernels.....	50
Butter fat.....	5
Röhmman's salt mixture.....	4
Lard.....	41

namely, to starvation, or to the presence of an injurious substance in the kernels. Evidence points to the latter, although mice deprived of all food except water die almost as soon as those on cotton seed kernels (tables 3 and 4). Nevertheless, control experiments revealed the fact that mice do not die when allowed

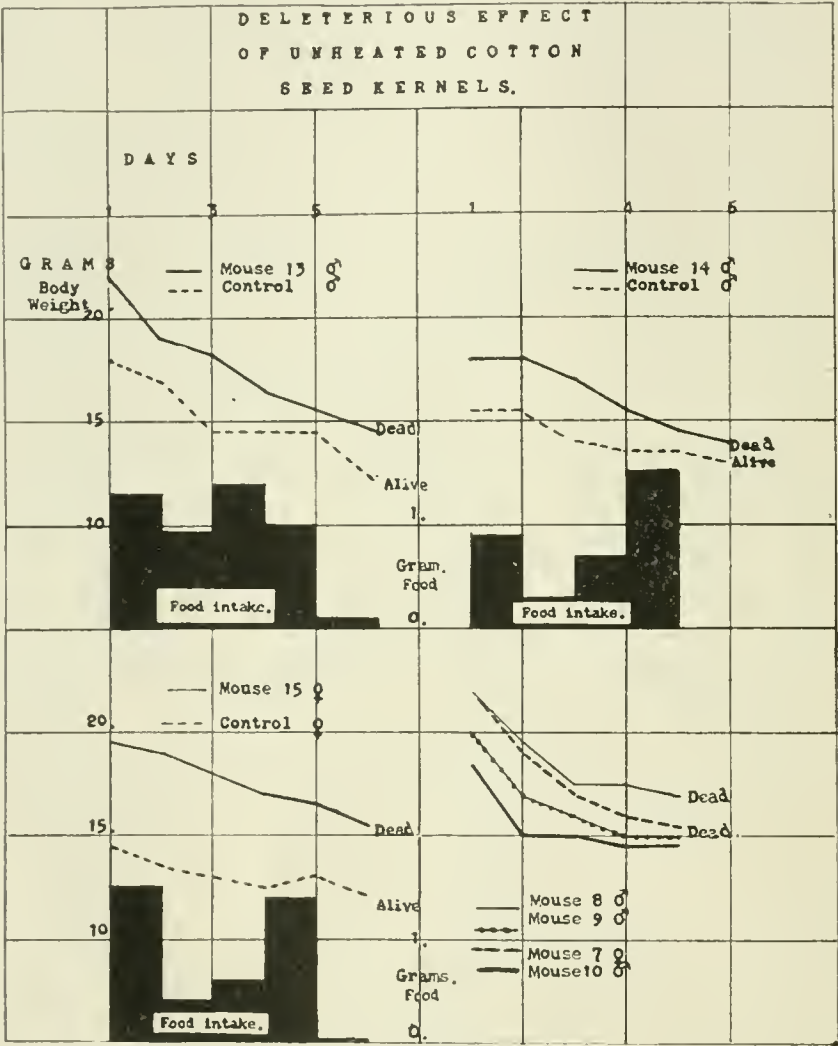


CHART 3

to consume a similar quantity of a non-toxic control cotton seed meal I ration (chart 3). Such results would suggest that cotton seed kernels contain *a something* that is unpalatable and toxic to mice. Similar results on rats have been reported by Osborne and Mendel (1917).

Cotton seed kernels combined with other products

Cotton seed *kernels* not only have a depressing effect upon the appetite of rabbits, guinea-pigs, pigeons, and albino mice, accompanied by a rapid decline in body weight, but are fatal to them. Similar results are obtained when cotton seed kernels

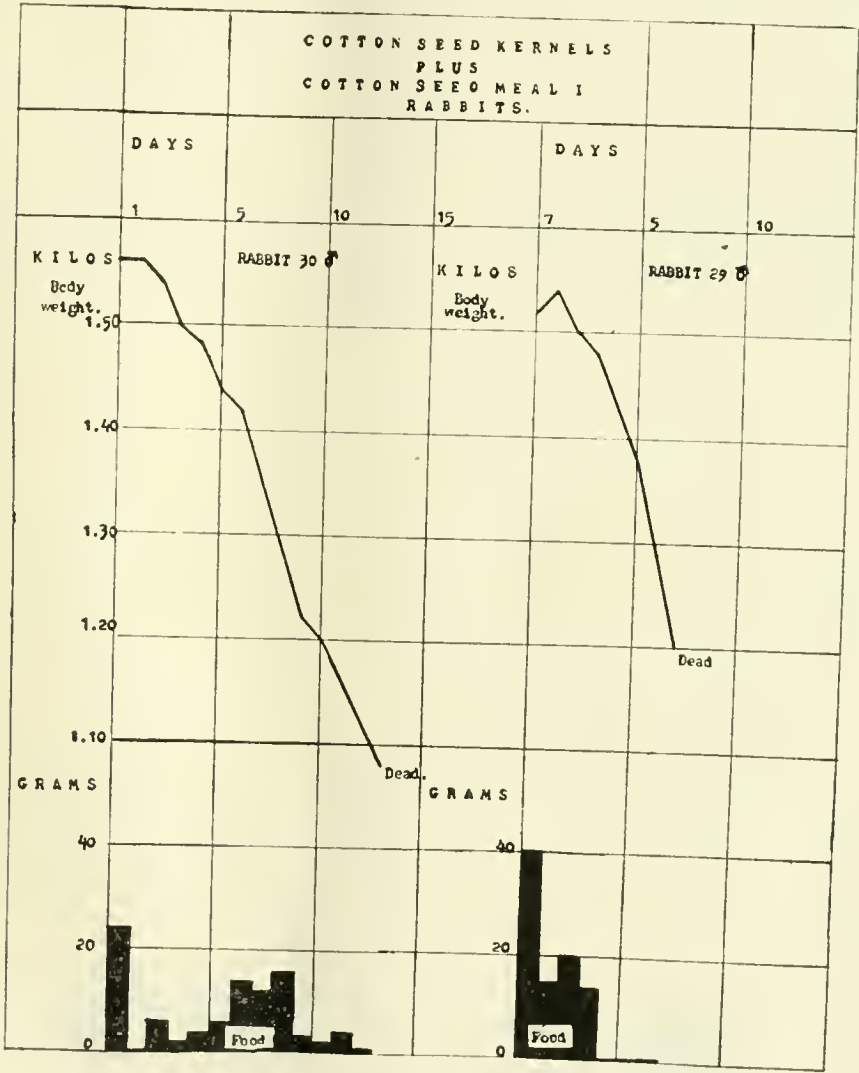


CHART 4

are incorporated with other food mixtures. Cotton seed meal I had not proved harmful to pigeons during a period of forty-seven days, but when it was combined with equal proportions of cotton seed kernels of the "Lone Star" variety and fed to pigeons, the mixture was partly ejected after each feeding, followed by a marked loss in body weight and death on the thirty-seventh day.

Other animals were fed on the same food mixture with similar results. Guinea-pig 4♂ died on the fourth day after eating 18 grams of the deleterious diet. Rabbit 30♂ died in thirteen days after a food intake of 85 grams. Such results obviously demonstrate the ill effects of a mixture of cotton seed kernels and cotton seed meal I. Cotton seed kernels combined with

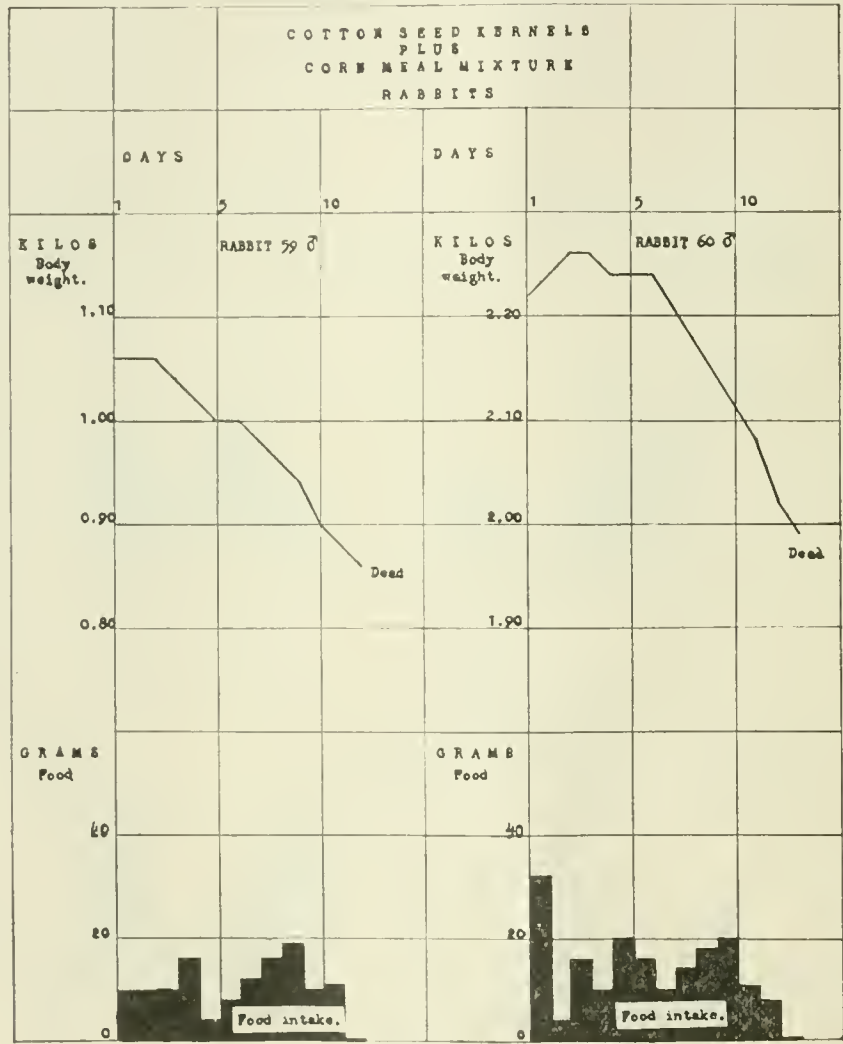


CHART 5

autoclaved cotton seed meal and also the corn meal mixture (1:1) had a similar depressing effect upon the appetite of rabbits, followed by loss in body weight and finally death (see charts 4, 5).

Mice fed on a ration containing 25 per cent of cotton seed kernels develop toxic symptoms. This supplemented diet containing:

	<i>per cent</i>
Cotton seed kernel.....	25
Cotton seed meal I.....	25
Butter fat.....	5
Röhmman's salt mixture.....	4
Lard.....	41

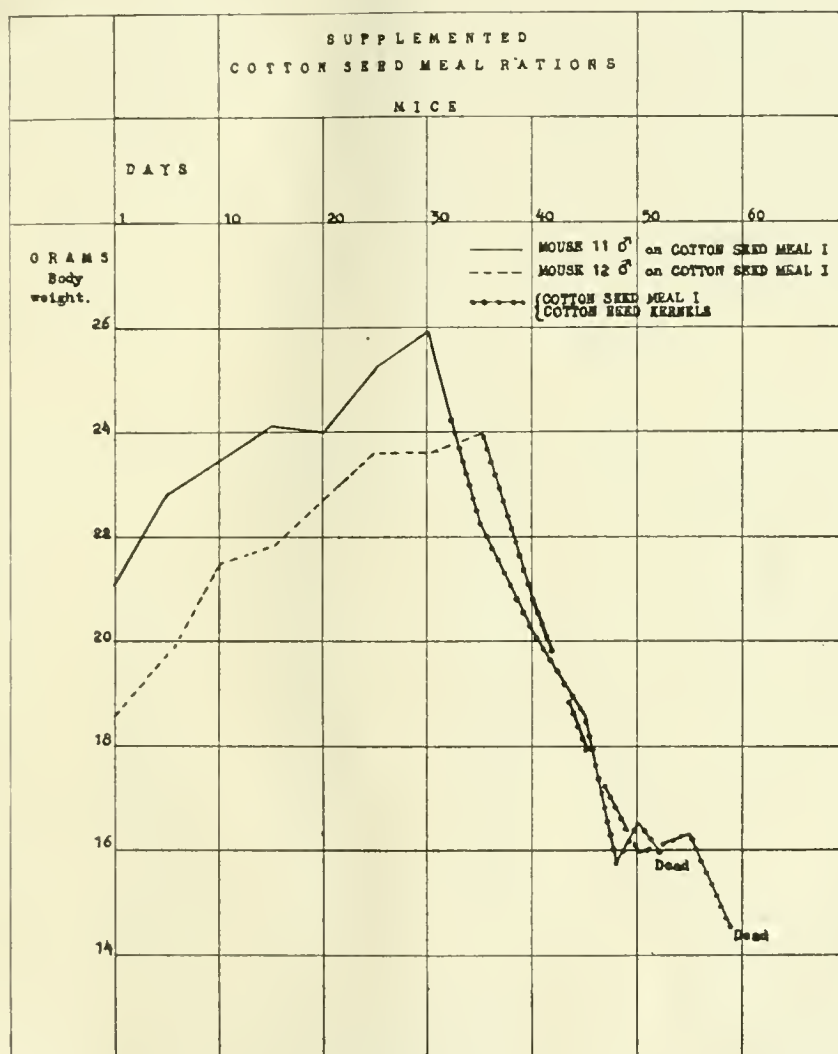


CHART 6

was fatal to mice, death occurring in thirteen to twenty days after a rapid decline in body weight. Even 25 per cent of cotton seed kernels in a diet otherwise adequate was fatal to mice. All of these observations are supplementary to those made on cotton seed kernels alone (chart 6).

FEEDING OF COTTON SEED MEALS

Animals decline on cotton seed meal

To determine the possible responsibility of inanition for the untoward results of the cotton seed meal injury careful records of the food intake were made. Cotton seed meal I¹⁰ of a bright color, and fine texture was purchased in a large feed store as a representative of the commercial products sold for feeding horses, cattle, swine, chickens, etc. It was fed to rabbits, the palatability being increased by the addition of molasses and 50 grams of cabbage per day as has previously been described. All rabbits declined rapidly as can be observed in table 5.

TABLE 5

Rabbits on cotton seed meal I

RABBIT	DURATION OF LIFE	ORIGINAL WEIGHT	FINAL WEIGHT	LOSS	
	<i>days</i>	<i>kilos</i>	<i>kilos</i>	<i>kilos</i>	<i>per cent</i>
43 ♂	17	2.14	1.50	0.64	30
44 ♂	12	1.78	1.38	0.40	22
45 ♂	12	1.86	1.54	0.32	17
46 ♂	15	1.90	1.34	0.56	29
47 ♂	15	1.86	1.36	0.50	27
48 ♂	14	2.22	1.56	0.66	29
50 ♂	12	2.14	1.84	0.30	14
51 ♂	9	2.20	1.90	0.30	23
52 ♂	21	2.32	1.60	0.72	31
Averages	14.2	2.04	1.56	0.49	24

¹⁰ Attached to the sack of cotton seed meal was a tag with the following analysis:

	<i>per cent</i>
Protein.....	not less than 36.0
Fat.....	not less than 5.0
Ammonia.....	not less than 7.0
Nitrogen.....	not less than 5.75
Carbohydrate.....	not less than 30.0
Fiber.....	not more than 15.0

“Puritan Cotton Seed Meal” sold by J. E. Soper Co., Boston, Mass.

How much cotton seed meal I will animals eat?

Inasmuch as rabbits decline very rapidly and finally die on cotton seed meal I, it was desired to learn whether or not death resulted from starvation. In order to do this the actual food intake was determined. Eleven rabbits ate quite liberally of the cotton seed meal moistened with molasses at first, after which they became sick of it and ate little or none the last few days of life. Moreover the cabbage, 50 grams of which were fed daily, was refused the last day or two of life although in some

TABLE 6
Rabbits on cotton seed meal I

RABBIT	DURATION OF LIFE	FOOD INTAKE	ORIGINAL WEIGHT	FINAL WEIGHT	LOSS	
	<i>days</i>	<i>grams</i>	<i>kilos</i>	<i>kilos</i>	<i>kilos</i>	<i>per cent</i>
1 ♀	11	190	1.61	1.20	0.41	25
3 ♂	13	99	1.90	1.36	0.54	28
4 ♂	14	155	1.80	1.32	0.48	26
5 ♂	9	100	1.70	1.23	0.47	27
6 ♂	14	158	1.82	1.23	0.59	32
7 ♂	10	89	1.90	1.30	0.60	31
8 ♂	12	91	2.06	1.40	0.66	32
9 ♀	13	122	1.90	1.24	0.66	33
10 ♀	12	201	1.70	1.20	0.50	29
11 ♂	8	40	1.70	1.34	0.36	21
12 ♂	11	131	2.32	1.62	0.70	30
Averages	11.5	125	1.86	1.31	0.55	29

cases it was eaten, to some extent, within a few hours of death. The ill effects of the diet, in some instances, became evident on the fifth or sixth day, while in other instances, the onset came at a later period; decline was manifested by lack of appetite, rough hair coat, weakness, and at length by death.

The loss in body weight and the daily consumption of cotton seed meal are summed up in table 6 and illustrated in charts 7 and 8, selected from many similar tests.

Cotton seed meal I proved fatal to guinea-pigs. As a rule these animals refused the diet at first, and it was found advisable to feed them on corn and oats mixture until they became accus-

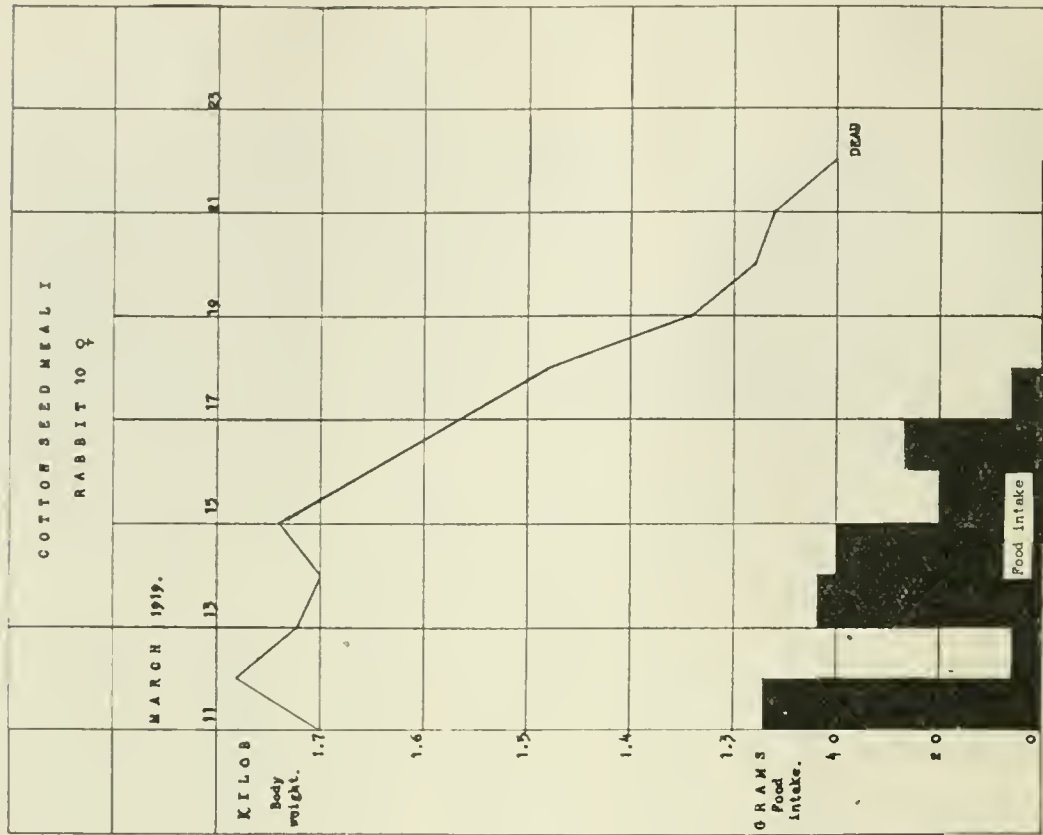


CHART 8

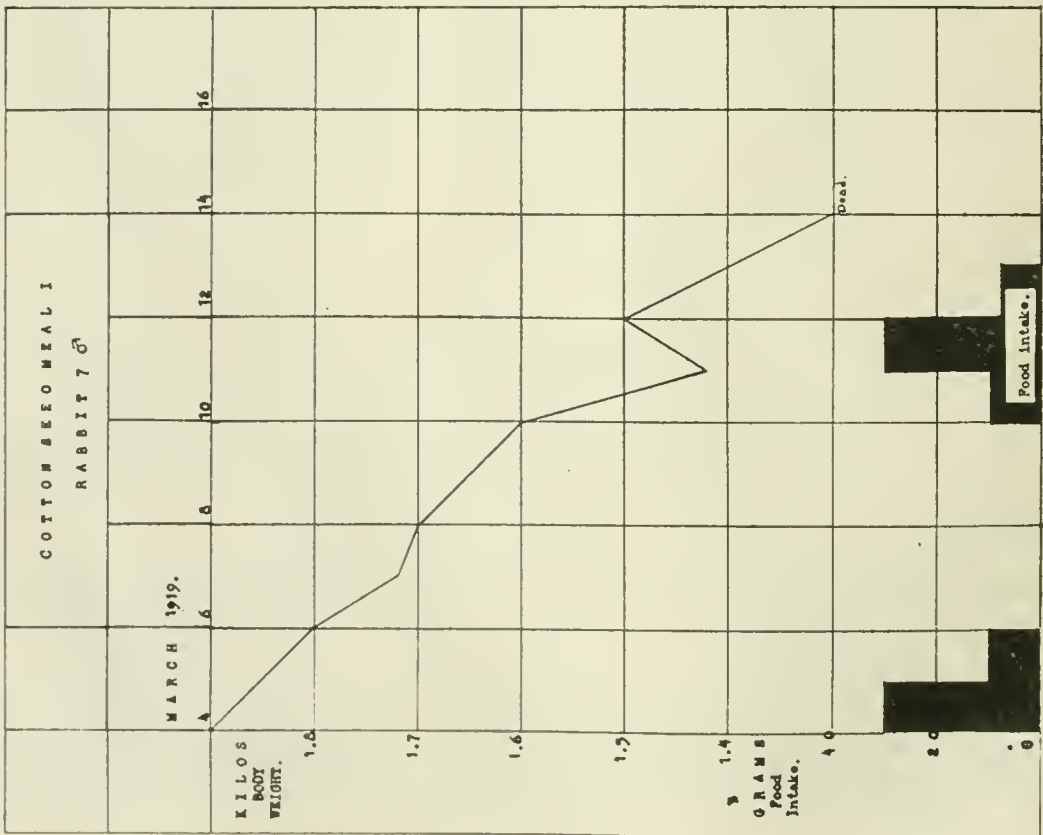


CHART 7

tomed to their new surroundings before starting the experimental diets. Usually they ate cotton seed meal I more freely, the length of life was longer, and there was a greater food intake per gram of body weight than in case of the rabbits. Guinea-pig I ♀ ate ravenously for twenty-one days, consuming 250 grams of cotton seed meal I with a loss of 18 per cent of its initial body weight; while guinea-pig 2 ♀ survived forty-one days, with a food intake of 580 grams and a loss of 23 per cent of body weight. Two guinea-pigs were fed like quantities of the control corn meal mixture to ascertain whether or not death could be attributed to inanition, but the results in charts 9 and 10 lead to the conclusion that this was not the cause. These results contribute to the evidence that cotton seed meal has toxic properties.

Two pigeons which had been fed cotton seed meal I for forty-seven days, although they appeared to be enjoying good health, were killed and autopsied. As this meal had been fatal to rabbits and guinea-pigs, it was thought that perhaps these birds would reveal the onset of cotton seed meal injury through a histological study of the internal organs. No pathological lesions were detected.

In order to make a complete comparable study of the effects of cotton seed meal I on all our experimental animals, mice also were fed this meal-molasses mixture. They survived from five to six days, a period which corresponds closely to that of starvation, in which case it was impossible to determine whether death was due to failure to eat, a deficient diet, or to the harmful effects of the food; firstly, because it was impossible to ascertain the food intake as mice tend to strew dry food materials on their feet; secondly, the period of time was too short to show accurately the rôle of a deficient diet; thirdly, post mortem examinations revealed nothing significant. Nevertheless, mice fed for five weeks on the control corn meal-molasses mixture gained in body weight and showed every sign of thrift. It is very probable that the cotton seed meal I, when fed as such, is distasteful to mice and that death is the result of inanition. This view is further substantiated by the fact that six mice, four males and two females, thrived for a period of six months on a supplemented cotton seed meal I diet.

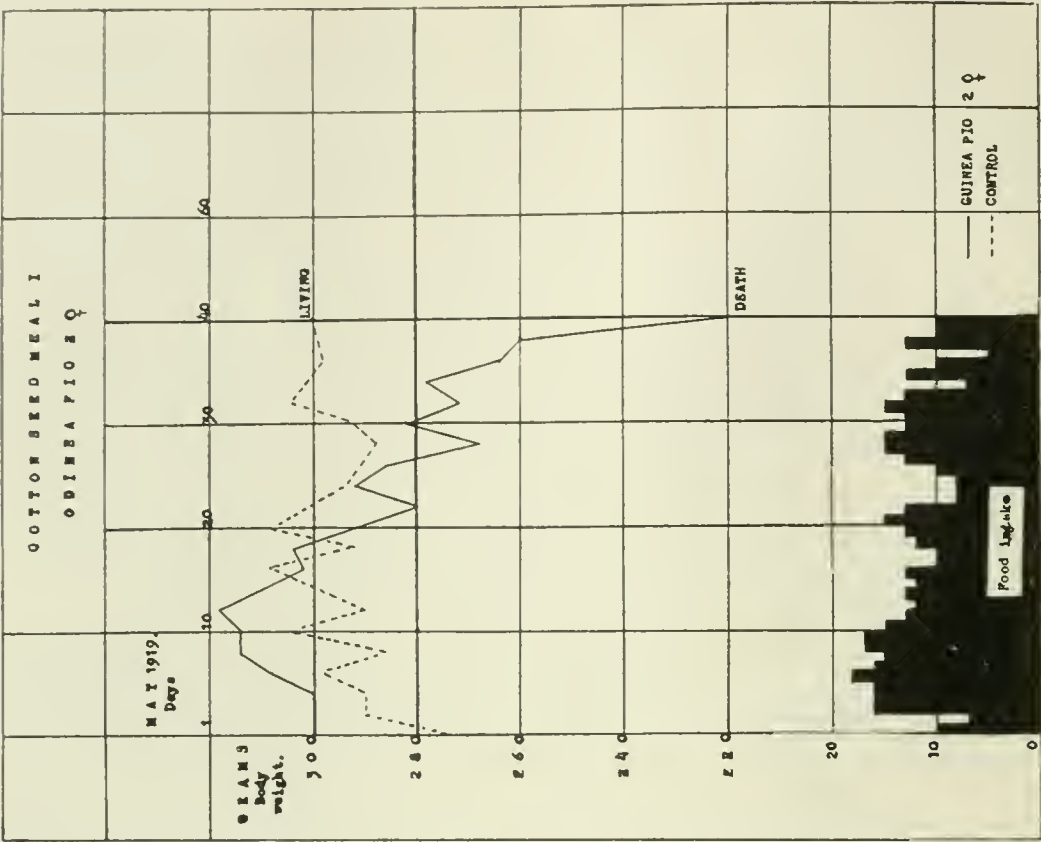


CHART 10

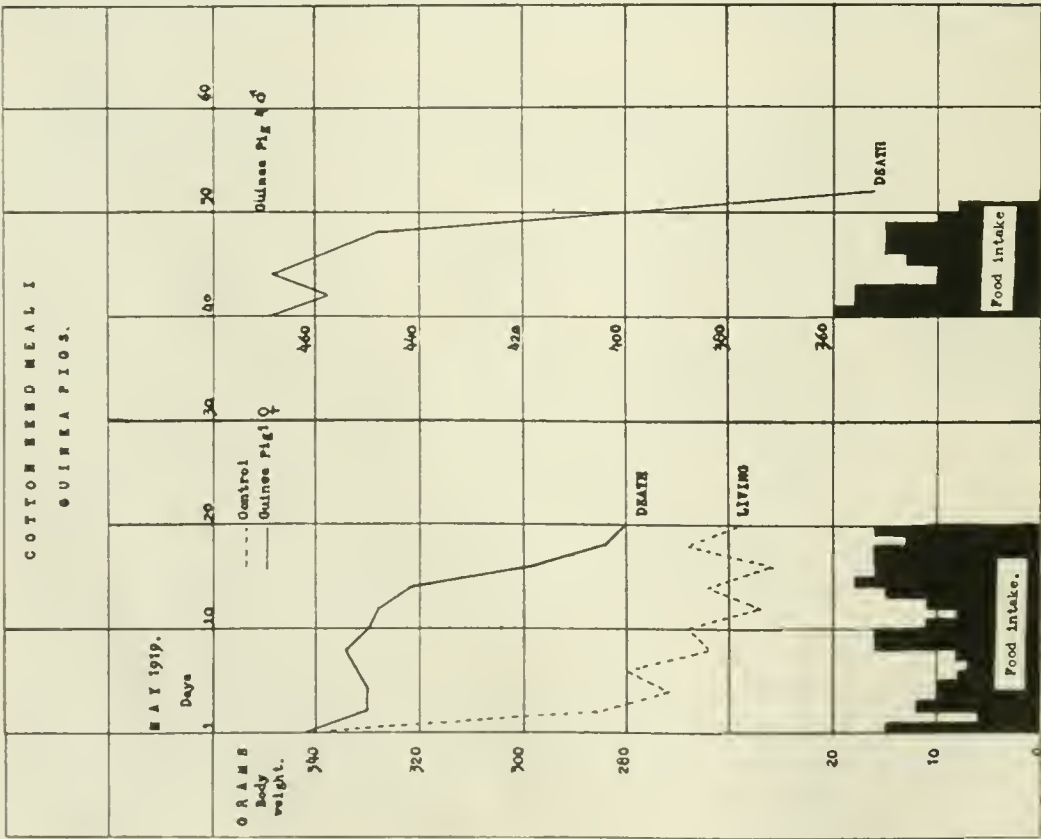


CHART 9

As a rule, the rabbits and guinea pigs developed immediate toxic symptoms on cotton seed meal I while pigeons and mice manifested little or no susceptibility.

Different samples of cotton seed meal vary

That cotton seed meal varies according to the condition of seed variety, soil, climate, and process of manufacture, has been established by the experience of several investigators and further verified in the present work. Different samples of cotton seed meal were found to vary in their immediate effects upon animals. The results of feeding cotton seed meal I have just been discussed. Cotton seed meal II¹¹ was purchased in the open market and fed to experimental animals, as has previously been described. Two rabbits, 58 ♂ and 61 ♂, lived fourteen and eighteen days, losing 29 and 34 per cent of their initial body weights, respectively. Control animals receiving similar amounts of food daily, were alive and had suffered only a slight loss in body weight at the conclusion of the experiment (chart 11). Statistics are given below for guinea-pigs and pigeons receiving the same ration.

From the results obtained, there can be no doubt as to the deleterious effects of cotton seed meal II upon rabbits, guinea-pigs and pigeons; this is further exemplified with mice, as can be observed in chart 12. Even though this meal was supplemented with butter fat and mineral salts, its ill effects were evident when mice were changed from cotton seed meal I ration to sample II; some mice died and others showed rapid decline after fifteen to thirty days. Such observations demonstrate that cotton seed meal II was actively toxic to all four species of animals.

¹¹ Danish brand—cotton seed meal. Guaranteed analysis:

	<i>per cent</i>
Protein.....	36.0
Fat.....	5.0
Crude fiber.....	15.0
Carbohydrate.....	25.0
Equivalent nitrogen.....	5.75

Manufactured for Humphreys-Goodwin Company, Memphis, Tenn.

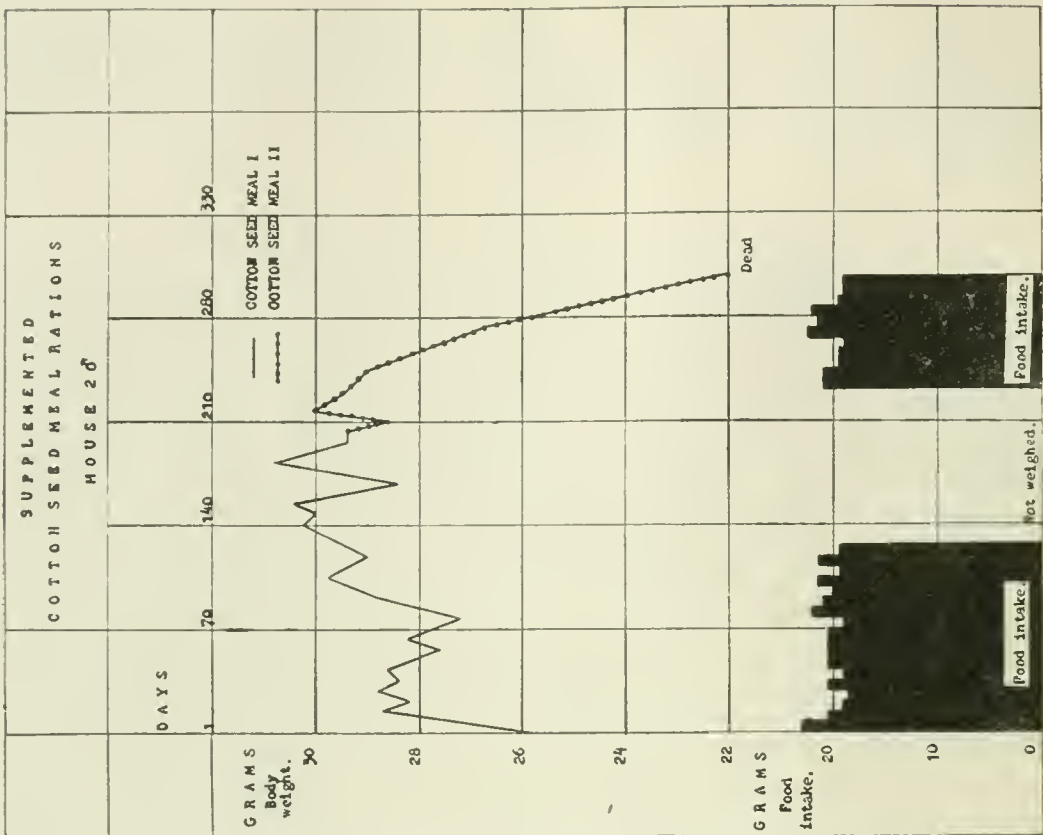


CHART 12

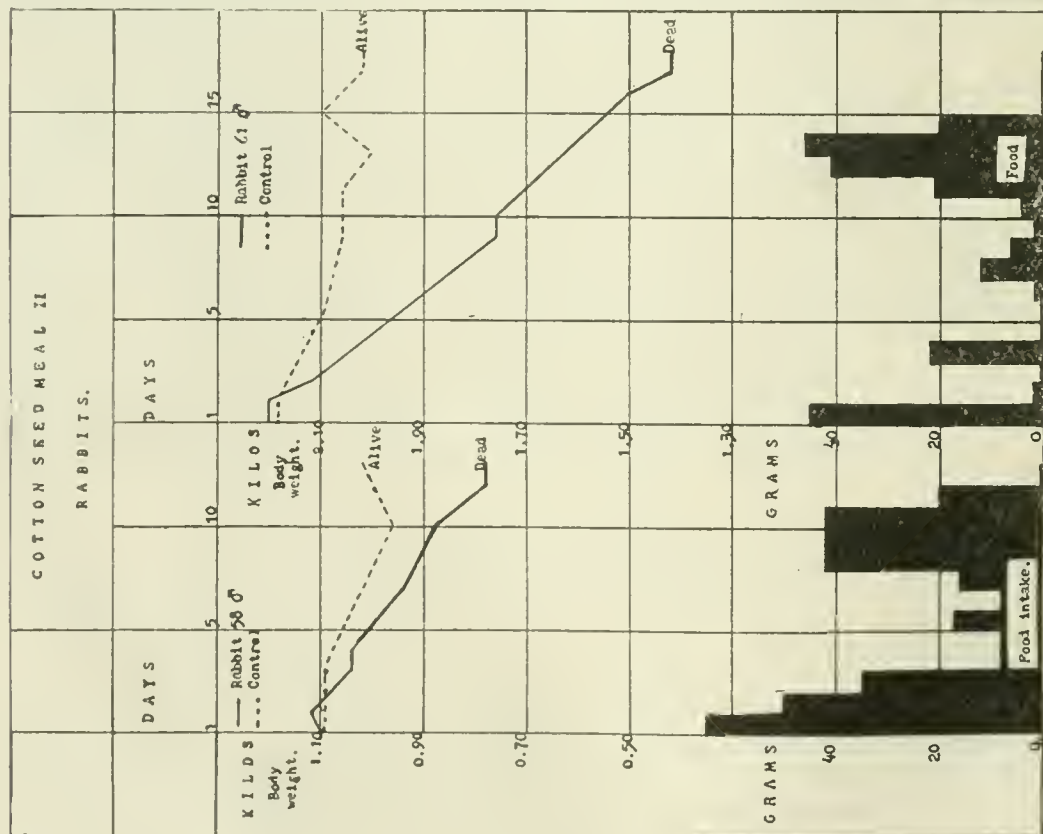


CHART 11

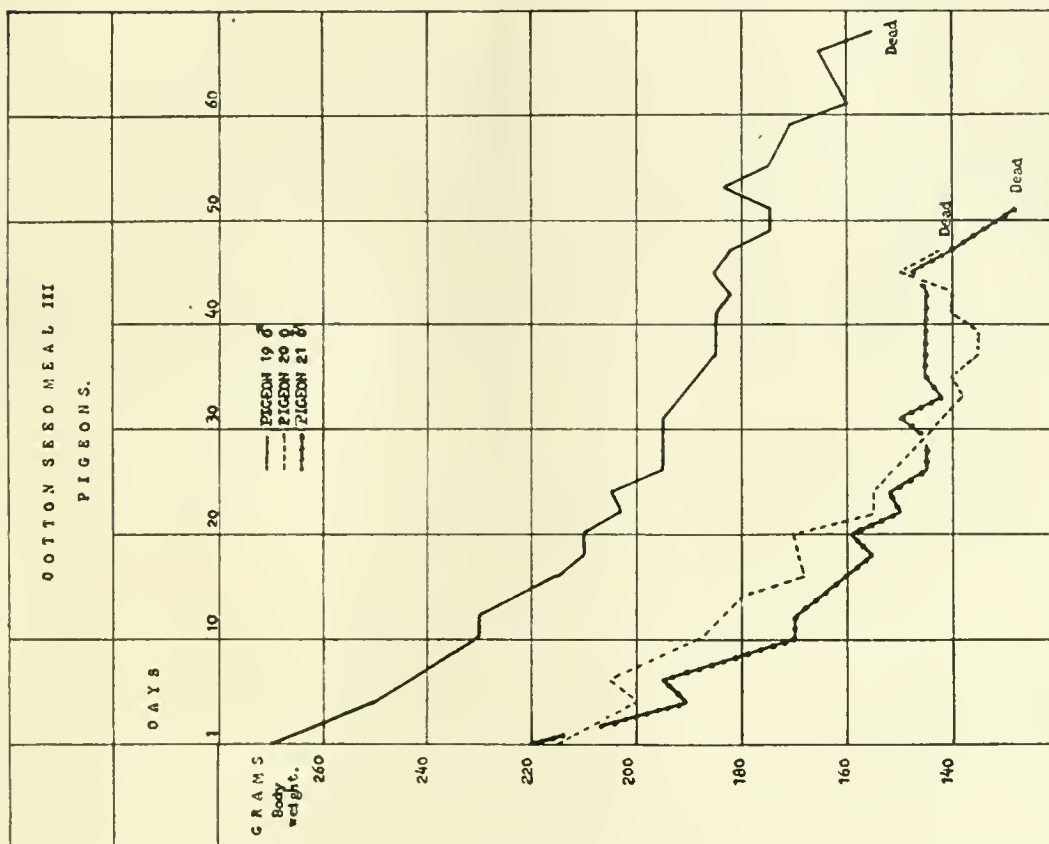


CHART 14

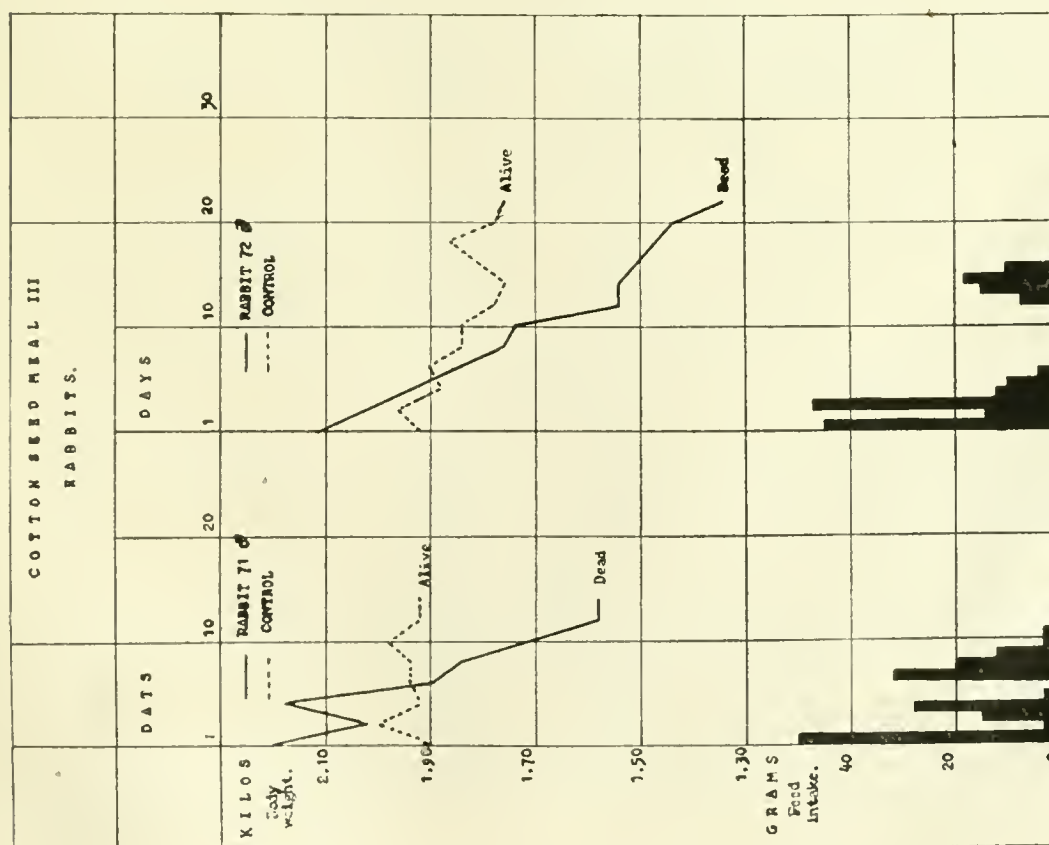


CHART 13

TABLE 7

Animals fed on cotton seed meal II

ANIMAL	DURATION OF LIFE	COTTON SEED MEAL II FOOD INTAKE	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	LOSS	
Guinea-pigs						
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i> *	<i>per cent</i>
9 ♂	13	89	550	430	120	21
10 ♂	14	70	410	300	110	27
11 ♀	14	87	275	200	75	27
12 ♀	25	126	270	174	96	35
Pigeons						
7	9	90	285	225	60	21
8	15	150	300	200	100	33
9	16	160	305	190	115	37
10	8	80	265	195	70	26

TABLE 8

Animals fed on cotton seed meal III

ANIMAL	DURATION OF LIFE	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	LOSS	
Rabbits					
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>
7 ♂	45	2400	1420	980	40
71 ♂	13	2200	1580	620	28
72 ♂	22	2120	1340	780	32
Guinea-pigs					
17 ♂	15	575	475	100	17
20 ♂	19	465	345	120	25
21 ♂	35	560	380	180	30
Pigeons					
19 ♂	80	270	150	120	44
20 ♀	47	215	143	72	33
21 ♂	51	220	128	92	41

Inasmuch as two samples of the same commodity had exhibited such different properties, a third variety, cotton seed meal III, was bought at a different feed store. Neither the brand, manufacturer, nor the analysis, are known, but it was a representative sample of a product which was being sold as live stock feed. A comparative study of its effects was made on three rabbits (chart 13), three guinea-pigs, and three pigeons (chart 14). The average span of life for three species was longer, as will be observed in tables 7 and 8.

TREATMENTS WHICH MAY INFLUENCE THE DELETERIOUS
PROPERTIES OF COTTON SEED MEAL

Heat as a detoxicating agent

Cotton seed meal I which has been demonstrated to be harmful to rabbits was rendered less injurious when subjected to heat for varying lengths of time under different conditions; but experiments show that even the most thoroughly heated meal was still slightly toxic to these animals. Mild treatment with dry heat had little effect upon the noxious properties; for rabbits when fed on cotton seed meal I which had been heated in an electric oven for six hours at 110°C., died just as soon as those on the unheated meal (see chart 15). Another sample steam heated in an Arnold sterilizer for a similar length of time, was rendered more palatable and the effects less harmful. The resulting material was consumed more freely by rabbits and the average length of life was longer than on the untreated meal (charts 16 and 17). Other lots of the same specimen of cotton seed meal, autoclaved for four hours under 20 pounds pressure, were eaten ravenously by the same animal, in most instances, with an increase in body weight for the first few days followed by a gradual decline, death finally ensuing at a much later period than on the untreated material (charts 18 and 19). Such results obviously lead to the conclusion that vigorous treatment with moist heat renders cotton seed meal I less harmful and more palatable to rabbits.

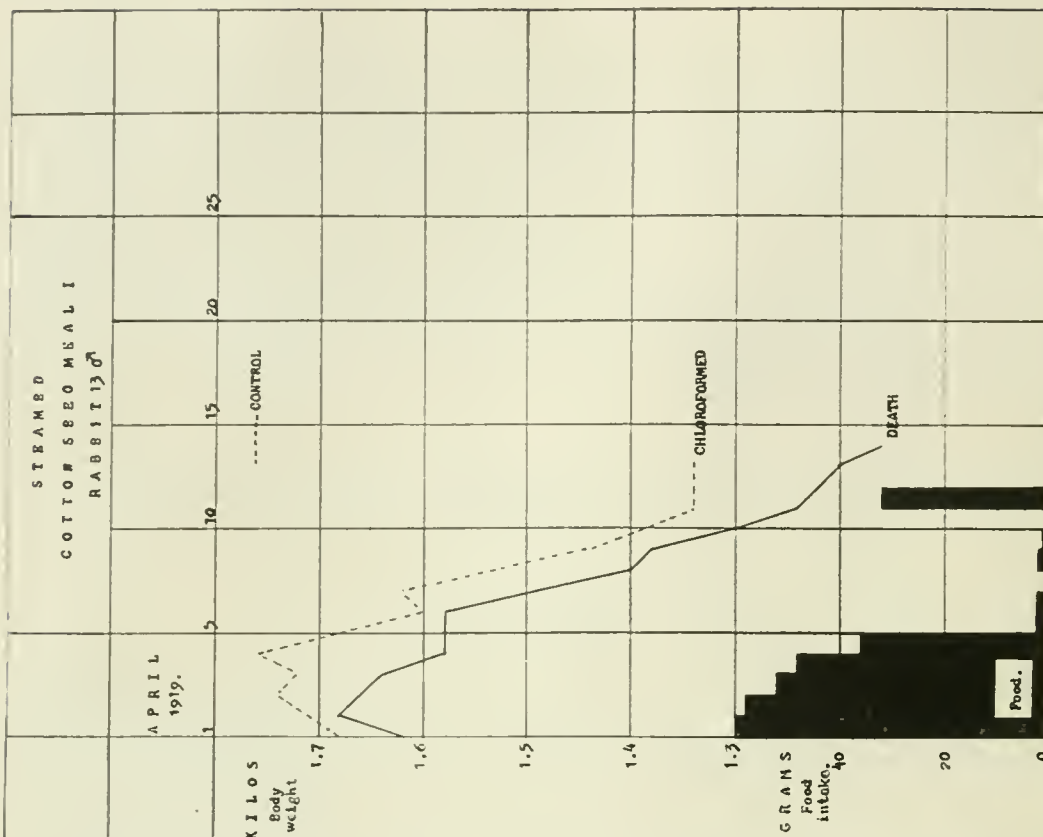


CHART 16

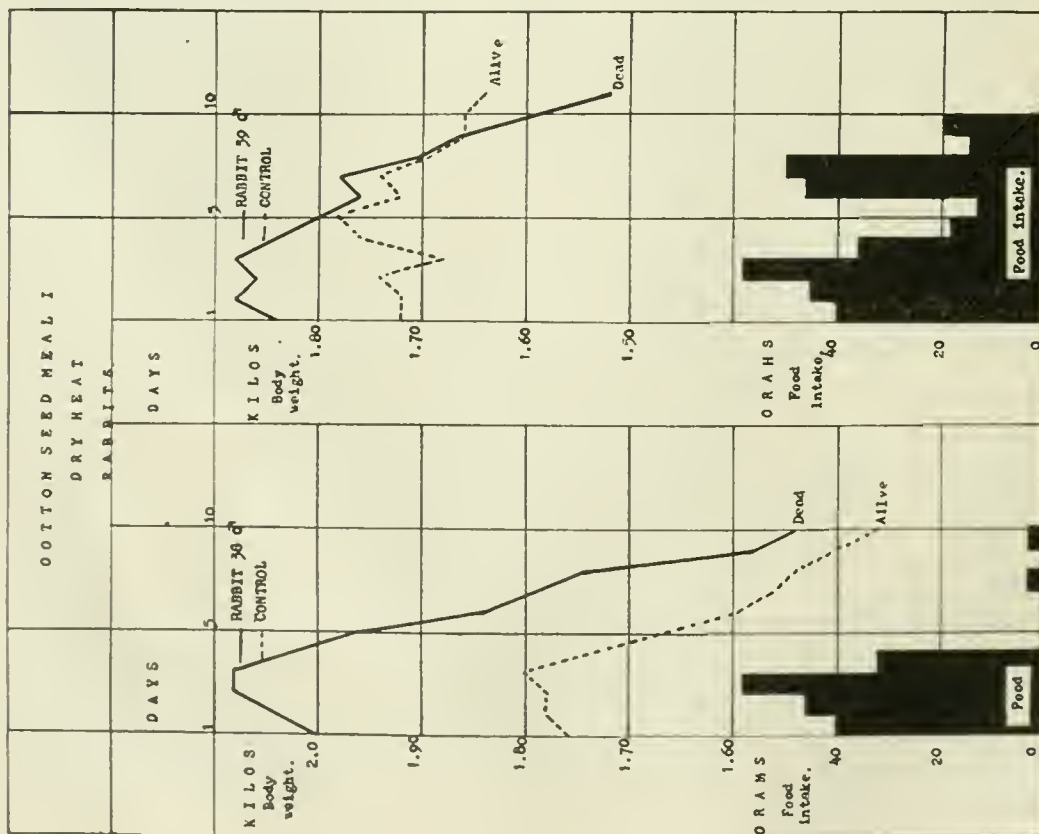


CHART 15

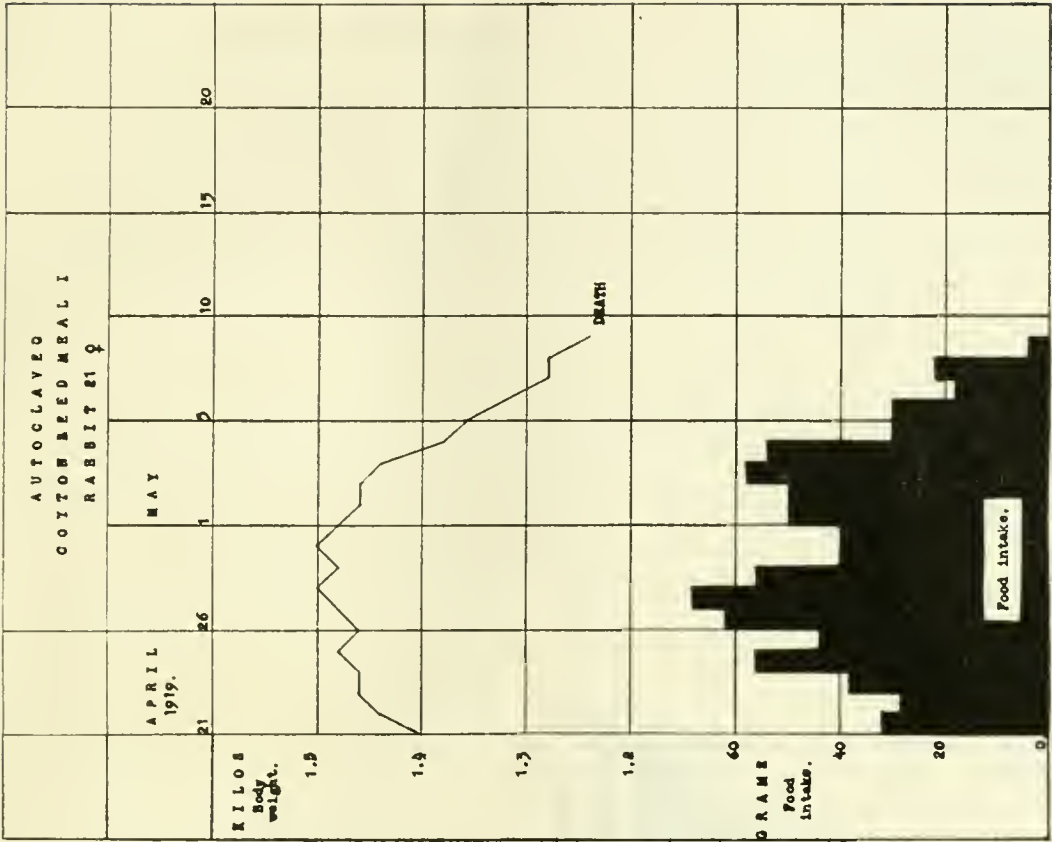


CHART 18

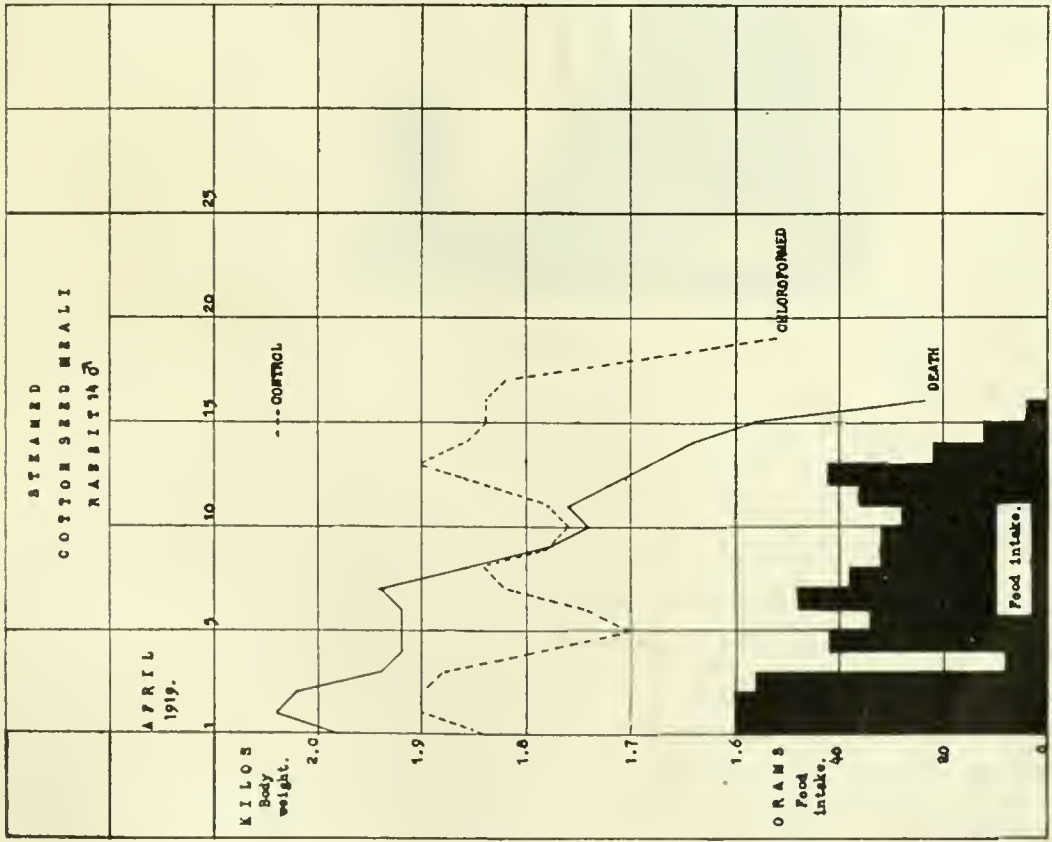


CHART 17

Results of these experiments on heated cotton seed meal are as shown in table 9.

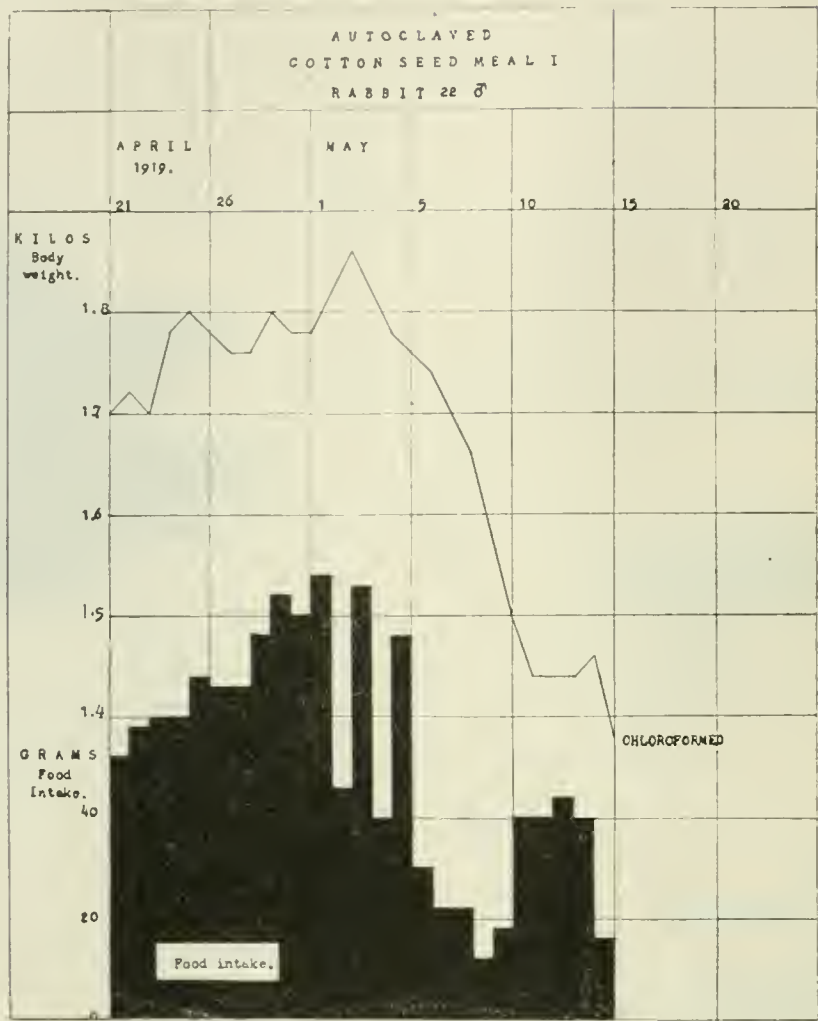


CHART 19

Extraction of cotton seed meal by ether

Cotton seed kernels have been successfully extracted with ether and the residual meal fed in a supplemented diet to rats without detrimental results (Osborne and Mendel, 1917), (Withers and Carruth, 1918b). Ether extraction of cotton seed meal is not always so efficacious. Withers and Carruth (1915b) having demonstrated that ether extraction does not render cotton seed meal non-toxic for rabbits, their experiments were repeated and the same results found to be correct for rabbits which were

also fed the ether extract and residue of different samples of this food stuff for comparable studies. Samples of both cotton seed meals I and II were extracted thoroughly with gasoline to remove the fat, and after the removal of the solvent by evaporation the residual material was subjected to ether extraction. One specimen, cotton seed meal I, was kept under ether from

TABLE 9

Rabbits on heated cotton seed meal I

RABBIT	DURATION OF LIFE	FOOD INTAKE	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	LOSS	
A. Cotton seed meal I heated in electric oven six to ten hours at 110°C.						
	<i>days</i>	<i>grams</i>	<i>kilos</i>	<i>kilos</i>	<i>kilos</i>	<i>per cent</i>
36 ♂	5	31	1.56	1.16	0.40	25
38 ♂	11	96	2.00	1.54	0.46	23
39 ♂	12	300	1.84	1.52	0.32	17
42 ♂	8	128	1.62	1.22	0.40	24
55 ♂	20		1.80	1.26	0.54	30
56 ♂	16		1.80	1.26	0.54	30
57 ♂	12		1.70	1.16	0.54	31
B. Cotton seed meal I heated in Arnold sterilizer for six hours						
13 ♂	14	297	1.62	1.06	0.56	35
14 ♂	16	547	1.98	1.42	0.56	28
15 ♂	16	522	2.30	1.72	0.58	25
16 ♂	13	264	2.00	1.40	0.60	30
C. Cotton seed meal autoclaved four hours at 20 pounds pressure						
21 ♀	19	775	1.40	1.24	0.16	11
22 ♂	25	1100	1.70	1.38	0.32	18
23 ♂	37	1680	2.12	1.58	0.54	25
24 ♂	37	3195	2.30	2.20	0.10	4
37 ♂	10	386	1.46	1.20	0.26	17
40 ♂	13	593	1.86	1.38	0.48	25
41 ♂	23	562	1.58	1.20	0.38	23

May until October, the ether being changed six times; then to insure a complete process, the remaining meal was extracted in a Soxhlet apparatus until the solvent drained off colorless. A similar treatment was given cotton seed meal II. All the ether solutions for each specimen of meal were collected, the ether distilled off, and the remaining dark red liquid fed with the

control corn meal mixture. The results of the feeding experiments with gasoline-ether extracted cotton seed meals and the ether extractives of the same are tabulated in table 10.

TABLE 10

ANIMAL	DURATION OF EX- PERIMENT	COTTON SEED MEAL INTAKE	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	REMARKS
A. Extracted cotton seed meal I					
	<i>days</i>	<i>grams</i>	<i>kilos</i>	<i>kilos</i>	
Rabbit 62 ♂.....	22	300	2.02	1.30	Dead
Rabbit 63 ♂.....	30	700	2.10	1.32	Dead
B. Ether extract of cotton seed meal I					
Rabbit 66 ♂.....	21	1000*	1.84	1.88	Alive
Rabbit 67 ♂.....	21	1000*	1.30	1.36	Alive
C. Extracted cotton seed meal II					
			<i>grams</i>	<i>grams</i>	
Pigeon 13 ♂.....	17	100	320	175	Dead
Pigeon 14 ♀.....	17	100	285	180	Dead
Pigeon 15 ♂.....	15	150	355	195	Recovered on corn meal
Pigeon 16 ♀.....	15	150	325	215	Recovered on corn meal
Guinea-pig 13 ♀...	22	263	397	320	Recovered on corn meal
Guinea-pig 14 ♂...	10	14	383	293	Dead
Guinea-pig 15 ♀...	22	251	403	299	Recovered
Guinea-pig 16 ♂...	22	269	400	305	Recovered
D. Ether extract of cotton seed meal II on corn meal mixture					
Pigeon 22 ♀.....	36	360*	225	255	Alive
Pigeon 23 ♂.....	36	360*	265	280	Alive
Pigeon 24 ♀.....	36	360*	295	300	Alive
Pigeon 25 ♂.....	36	360*	350	350	Alive
Guinea-pig 6 ♂....	29	335*	365	260	Dead

* Equivalent amount of cotton seed meal I.

COTTON SEED MEAL INJURY IS NOT ATTRIBUTABLE TO INANITION

In most instances animals eat small quantities of cotton seed meal when it serves as the chief supply of food. Inasmuch as this is true, cotton seed meal death might be attributed to starvation, as well as to a deficiency or to the toxic properties of the meal. Carefully regulated experiments, in which animals receive

daily the same quantities of a control food mixture as that which the cotton seed meal victim devoured, demonstrate that death is not the result of inanition but is due to some other factor. Rabbits fed on cotton seed meal which had been heated in an Arnold sterilizer for six hours ate liberally but lost in body weight and finally died; while animals eating similar restricted quantities of the control diet lost weight but were alive and in good health at the conclusion of the experiment.

Rabbit 13 ♂ lived only fourteen days with an intake of 297 grams of the heated cotton seed meal and a loss of 34 per cent of its initial body weight; a control, rabbit 18 ♀, consuming the same quantity of corn meal mixture lost only 20 per cent of its body weight, and was thrifty at the end of fourteen days when it was killed and carefully examined for any pathological lesions which might occur on a limited diet. No deviations from the normal could be detected. Rabbit 14 ♂ survived for sixteen days during which it ate 547 grams of heated cotton seed meal I and lost 23 per cent of the body weight; its control lost only 4 per cent in body weight during the sixteen days. This rabbit was then deprived of all food except water until it had lost 23 per cent of its weight; it was killed and post mortem examination made but no characteristic pathological changes were found. Chart 20 shows similar results for rabbit 16 ♂ and control rabbit 17 ♀. Five hundred and twenty-two grams of cotton seed meal proved lethal for rabbit 15 ♂ on the sixteenth day. It lost 23 per cent of its original body weight while the control lost only 2 per cent. After the sixteenth day, the control rabbit 20 ♂ was starved (chart 21), death occurring fourteen days later. Upon examination no abnormalities could be detected in any of the internal organs, either grossly or in section.

Inanition can not be ascribed as the sole cause of the death of any of these animals fed on cotton seed meal ration; neither similar quantities of a control diet nor starvation produce any of the characteristic symptoms which result from cotton seed meal injury.

More extensive experiments were conducted on different species of animals on a variety of cotton seed meal rations in order to verify the above hypothesis. A synopsis is given in table 11.

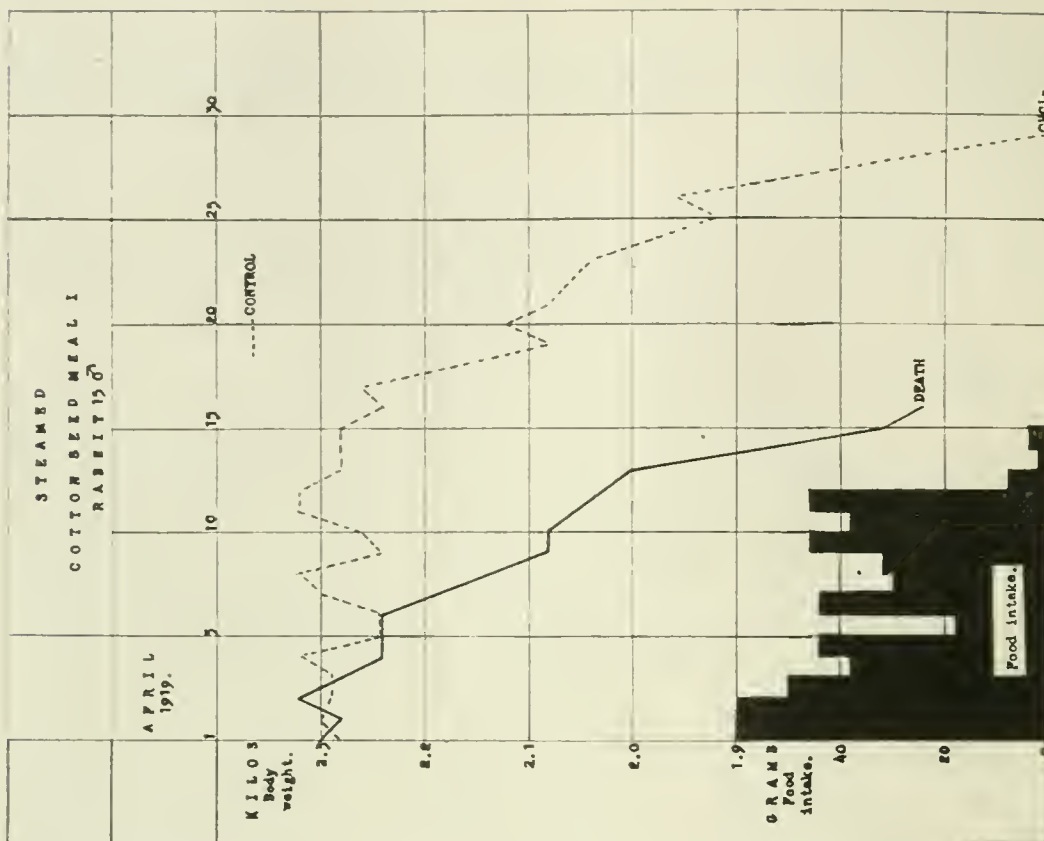


CHART 21

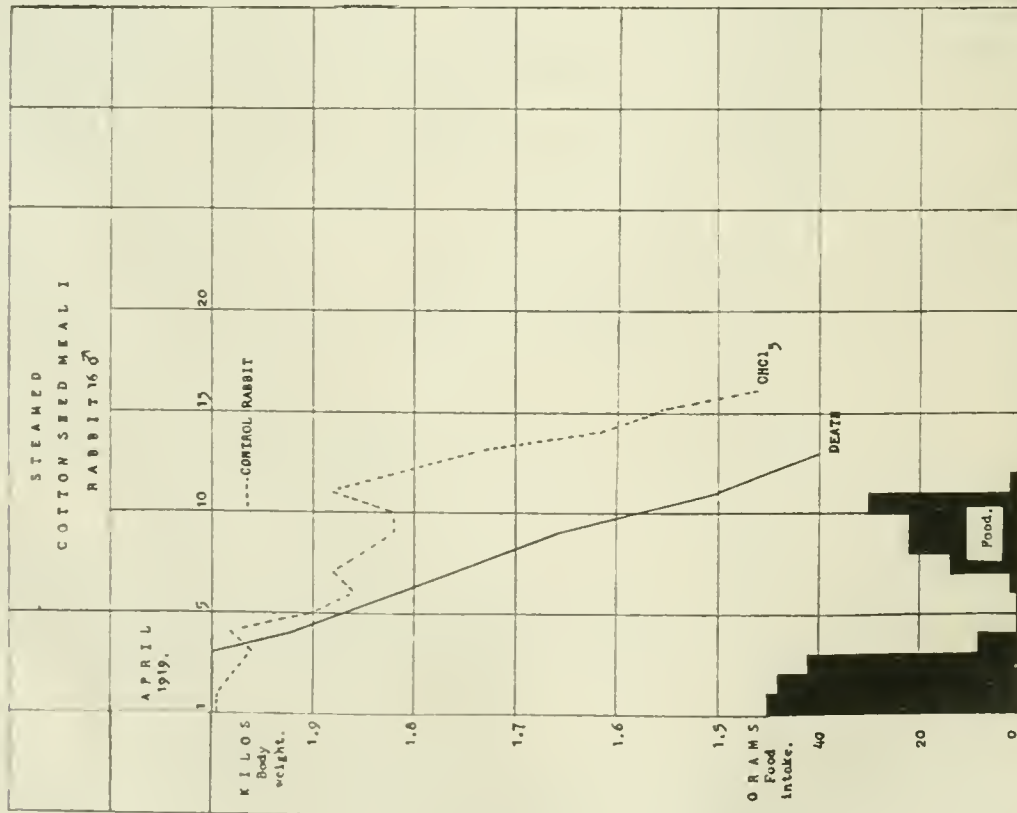


CHART 20

TABLE 11

Animals fed on cotton seed meal with controls

ANIMAL	DURATION OF EXPERI- MENT	FOOD INTAKE	ORIGINAL BODY WEIGHT	FINAL BODY WEIGHT	LOSS	REMARKS
A. Untreated cotton seed meal I						
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	
Guinea-pig 1 ♀	21	248	343.0	280.0	18	Dead
Control 4 ♂	21	248	520.0	460.0	15	Alive
Guinea-pig 2 ♀	41	580	300.0	230.0	23	Dead
Control 3 ♀	41	580	275.0	300.0	+9	Alive
B. Ether extracted cotton seed meal I						
			<i>kilos</i>	<i>kilos</i>		
Rabbit 25 ♂	22	265	2.04	1.48	27	Dead
Control 32 ♂	22	265	2.12	1.54	27	Alive
Rabbit 26 ♂	7	168	1.84	1.62	11	Dead
Control 29 ♂	7	168	1.86	1.66	10	Alive
C. Cotton seed meal I heated in Arnold sterilizer for six hours						
Rabbit 13 ♂	14	297	1.62	1.06	34	Dead
Control 18 ♀	14	297	1.60	1.34	16	Alive
Rabbit 14 ♂	16	547	1.98	1.42	28	Dead
Control 19 ♀	16	547	1.90	1.84	3	Alive
Rabbit 15 ♂	16	522	2.30	1.72	20	Dead
Control 20 ♂	16	522	2.08	1.94	6	Alive
Rabbit 16 ♂	13	264	2.00	1.40	30	Dead
Control 17 ♀	13	264	1.70	1.62	4	Alive
D. Cotton seed meal I heated in electric oven for eight hours at 110°C.						
Rabbit 36 ♂	5	31	1.56	1.16	25	Dead
Control 29 ♂	5	31	1.82	1.60	12	Alive
Rabbit 42 ♂	8	128	1.62	1.22	24	Dead
Control 30 ♂	8	128	1.68	1.56	7	Alive
Rabbit 38 ♂	11	96	2.00	1.54	23	Dead
Control 31 ♂	11	96	1.76	1.46	17	Alive
Rabbit 39 ♂	12	300	1.84	1.52	17	Dead
Control 32 ♂	12	300	1.72	1.66	3	Alive

TABLE 11—Continued

ANIMAL	DURATION OF EXPERI- MENT	FOOD INTAKE	ORIGINAL BODY WEIGHT	FINAL BODY WEIGHT	LOSS	REMARKS
E. Cotton seed meal II						
	<i>days</i>	<i>grams</i>	<i>kilos</i>	<i>kilos</i>	<i>per cent</i>	
Rabbit 58 ♂.....	14	241	1.10	0.78	20	Dead
Control 75 ♂.....	14	241	1.10	1.02	7	Alive
Rabbit 61 ♂.....	18	223	2.20	1.44	34	Dead
Control 76 ♂.....	18	223	2.18	2.02	7	Alive
			<i>grams</i>	<i>grams</i>		
Pigeon 7 ♂.....	9	90	285.0	225.0	21	Dead
Pigeon 8 ♂.....	15	150	300.0	200.0	33	Dead
Pigeon 9 ♂.....	15	150	305.0	190.0	34	Dead
Pigeon 10 ♂.....	8	80	265.0	195.0	26	Dead
Control 5 ♀.....	15	150	270.0	245.0	9	Alive
F. Cotton seed meal III						
Guinea-pig 23 ♀.....	12	114	296.0	238.0	19	Dead
Control 8 ♀.....	12	114	310.0	315.0	+17	Alive
			<i>kilos</i>	<i>kilos</i>		
Rabbit 71 ♂.....	14	169	2.20	1.58	28	Dead
Control 59 ♂.....	14	169	1.90	1.92	+1	Alive
Rabbit 72 ♂.....	23	178	2.12	1.34	32	Dead
Control 59 ♂.....	23	178	1.92	1.78	7	Alive
G. "Lone Star" cotton seed kernels						
Rabbit 31 ♂.....	5	17	1.46	1.18	19	Dead
Control.....	5	17	1.56	1.16	25	Alive
Rabbit 32 ♂.....	12	38	1.70	1.32	23	Dead
Control.....	12	38	1.74	1.32	21	Alive
Rabbit 33 ♂.....	32	336	3.26	2.02	38	Dead
Control.....	32	336	2.90	1.76	39	Alive
Rabbit 35 ♂.....	26	96	2.50	1.82	27	Dead
Control.....	26	96	2.30	1.52	33	Alive
			<i>grams</i>	<i>grams</i>		
Pigeon 8506 ♀.....	20	435	263.0	160.0	39	Dead
Pigeon 11 ♂.....	11	110	307.0	223.0	27	Dead
Pigeon 12 ♂.....	12	120	265.0	170.0	35	Dead
Control.....	20	200	280.0	270.0	3	Alive
Guinea-pig 3 ♀.....	5	30	293.0	252.0	14	Dead
Control 8 ♀.....	5	30	318.0	290.0	8	Alive

TABLE 11—*Concluded*

ANIMAL	DURATION OF EXPERI- MENT	FOOD INTAKE	ORIGINAL BODY WEIGHT	FINAL BODY WEIGHT	LOSS	REMARKS
H. Mice on supplemented cotton seed kernel food						
	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	
Mouse 13 ♂	6	4.8			35	Dead
Control 18 ♂	6	4.8			31	Alive
Mouse 14 ♂	6	3.4			23	Dead
Control 19 ♂	6	3.4			16	Alive
Mouse 15 ♀	6	3.9			20	Dead
Control 20 ♀	6	3.9			19	Alive

COTTON SEED MEAL INJURY NOT DUE TO A LACK OF WATER-SOLUBLE VITAMINE

Rommel and Vedder (1915), basing their conclusions on the similarity between the post mortem observations made on pigs that had died from cotton seed meal poisoning and those that had died from an exclusive diet of polished rice, believe that cotton seed meal poisoning is similar to beriberi in man. If this were true cotton seed meal injury could be averted by feeding cotton seed meal in combination with a food rich in water-soluble vitamine. According to the writers' experience such is not the case. It is to be reiterated that cabbage which was regularly fed with cotton seed meal, contains the three recognized types of vitamins; namely, fat-soluble A,¹² water-soluble B,¹³ and antiscorbutic, 10 grams being sufficient to avert scurvy in guinea-pigs.¹⁴ Yeast is rich in water-soluble vitamine and a very small amount will relieve a bird suffering from polyneuritis in a few hours. From the outset of the present work, all rabbits received 50 grams of cabbage, guinea-pigs received 20 grams, and the pigeons a generous amount finely chopped. Hence in no instance throughout the feeding of cotton seed meal rations to

¹² Steenbock, H., and Gross, E. G.: J. Biol. Chem., 1920, xli, 149.

¹³ Osborne and Mendel: J. Biol. Chem., 1919, xxxvii, 195.

¹⁴ Givens, Maurice H., and Cohen, Barnett: J. Biol. Chem., 1918, xxxvi, 130.

the above mentioned animals, was there a lack of water-soluble vitamine. Therefore death cannot be attributed to the lack of this food factor.

Experiments were also conducted in which large quantities of water-soluble vitamine were furnished by yeast: Guinea-pigs, 23 ♂ and 24 ♂, were fed on cotton seed meal II for nine days—in fact, until they had become weak and exhibited signs of rapid decline; one gram of dried brewer's yeast was then mixed in with daily meal diet. Yeast did not relieve the animals, as the former died on the twelfth day, the latter on the sixteenth day. This is evidence which leads to the conclusion that we are dealing with toxicity rather than vitamine deficiency.

In another series of experiments, guinea-pigs (guinea-pigs 13 ♀, 15 ♀ and 16 ♂) were given ether extracted cotton seed meal II until they became emaciated and ill; on the twenty-second day all were put on different diets. One-half the cotton seed meal was replaced by corn meal mixture for two animals, while the other received corn meal mixture alone. In addition to the corn meal mixture and extracted cotton seed meal, guinea-pig 15 ♀ received 1 gram of yeast daily. Throughout the experiment all animals received cabbage. On the thirty-fourth day, all animals had seemingly recovered to the same degree, although the body weights varied +10, -2, +3 per cent, respectively on the new diets. In other words the guinea-pig (15 ♀) that received an abundance of water-soluble vitamine recovered no better than the others, in fact, it actually lost while the others gained in weight.

Further results with pigeons exclude even a probability that there is a lack of water-soluble vitamine in the toxic cotton seed meal diet. Thus the standard corn meal mixture was subjected to heat in an autoclave for 4 hours under 20 pounds pressure. The resulting material was deficient in water-soluble vitamine for the pigeons developed polyneuritis in about fourteen days when fed exclusively upon it. Digestion became greatly impaired, the crops were distended with food, and a tendency to eject the undigestible food was exhibited in some cases while acute polyneuritis, in which the head was drawn back with

pronounced nervous excitability, was shown in others; the former condition will be spoken of as chronic polyneuritis since it was slower in developing, the latter as acute. After the health of the birds became greatly impaired, different samples of untreated cotton seed meal were administered in the usual way. All birds except one recovered. For example, pigeon 30 ♀ developed chronic polyneuritis while pigeon 31 ♀ gave evidence of the acute form; the autoclaved corn meal mixture diet was then replaced by the same quantity, 10 grams, of toxic cotton seed meal II. Pigeon 31 ♀ showed improvement on the second day, furthermore by the third day the digestion was good, and normal appearance had been regained. Pigeon 30 ♀ was down before the treatment was begun; after receiving cotton seed meal II it recovered more slowly, on the third day the crop was greatly distended with undigested food residue, there was improvement on the fourth day, and by the fifth day normal health had recurred.

Similar results were obtained with the toxic cotton seed meal III as a source of antineuritic vitamine. Pigeon 32 ♀ with acute polyneuritis showed improvement in four days on this meal. The different varieties of cotton seed meal used apparently contained a similar quantity of water soluble vitamine as all samples were effective to the same degree; however, it is found in less liberal quantities in cotton seed meal than in yeast. One gram of dry yeast was sufficient to relieve a pigeon of acute polyneuritis in a few hours, while it required from two to five days with 10 grams of cotton seed meal each day to completely cure an afflicted bird.

In order to confirm these results, two pigeons (pigeons 26 ♂ and 27 ♂) were fed polished rice until polyneuritis developed; 20 grams of cotton seed meal III were again efficacious in restoring these birds to health. Such results leave no doubt as to the antineuritic properties of cotton seed meals.

Osborne and Mendel (1917) found that cotton seeds contained water-soluble vitamine in considerable abundance; for rats grew satisfactorily on diets in which there was no other source of this accessory. Similar results have been obtained throughout our

investigation with albino mice. Experiments have previously been discussed in which mice lived for six months on a diet in which cotton seed meal I was the only source of water-soluble vitamine, a result confirmatory of those already discussed.

Effects of heating foods

The experiments, which have just been cited on the effects of heated corn meal mixture on pigeons, should be scrutinized before considering heat as a "detoxicating agent" for cotton seed meal. As we have noticed, autoclaving corn meal mixture for four hours under 20 pounds pressure, a treatment which rendered cotton seed meal I less injurious to rabbits, destroyed its antineuritic vitamine content; for pigeons were afflicted with polyneuritis when fed exclusively on it. The deleterious properties of cotton seed meal can be decreased by heat and it may be possible to render a toxic meal harmless by such a treatment. However, it must be borne in mind that the character of the food material may be changed by heat as is demonstrated by our experiments with heated corn meal on pigeons. Pigeons thrive on untreated corn meal for long periods of time but when the diet was changed to the same mixture, autoclaved, they developed polyneuritis.

If heat is used as the method for rendering cotton seed meal harmless, it must be borne in mind that the resulting material may need to be combined with a food rich in water-soluble vitamine at least. This is demonstrated in the experiments below.

The standard corn meal mixture which had been used with success throughout our investigation was subjected to the same treatment as was the cotton seed meal I, namely, autoclaved for four hours under 20 pounds pressure. It was then fed in combination with cabbage, 50 and 20 grams respectively, to rabbits and guinea-pigs. Rabbits 68♂ and 69♂ were kept for sixty-four days and guinea-pig 8♀ for eighty-two days, without any detrimental effects. None of our animals on heated cotton seed meal diets lived for so long a time. The palatability

of the corn mixture was unaffected by heating in so far as rabbits and guinea-pigs are concerned; for they ate approximately the same quantity as can be seen in table 12.

Two important facts are emphasized by the foregoing experiments. Firstly, the quantities of cabbage always fed along with cotton seed meals to rabbits and guinea-pigs throughout this investigation were alone sufficient to furnish enough water-soluble vitamine for the existence of these animals. Therefore, "cotton seed meal injury" cannot be attributed to the lack of this dietary essential in our experiments. Secondly, heated foods can be successfully fed if combined with other foods rich in water-soluble vitamine.

TABLE 12

ANIMAL	DURATION OF EXPERIMENT	AVERAGE FOOD CONSUMED DAILY	INITIAL BODY WEIGHT	FINAL WEIGHT
A. Animals on untreated corn meal mixture				
	<i>days</i>	<i>grams</i>	<i>kilos</i>	<i>kilos</i>
Rabbit 64 ♂.....	60	50	1.72	1.56
Rabbit 65 ♂.....	60	47	1.36	1.44
			<i>grams</i>	<i>grams</i>
Guinea-pig 8 ♀.....	60	13+	225.0	342.0
B. Animals on autoclaved corn meal mixture				
			<i>kilos</i>	<i>kilos</i>
Rabbit 68 ♂.....	60	42	2.20	2.08
Rabbit 69 ♂.....	60	42	1.84	1.82
			<i>grams</i>	<i>grams</i>
Guinea-pig 8 ♀.....	60	13±	340.0	300.0

CAN ANIMALS RECOVER FROM "COTTON SEED MEAL INJURY?"

The fate of an animal suffering from "cotton seed meal injury" seems to depend upon the severity of the attack or upon the extent to which the internal organs are affected. In some cases animals will recover when the noxious diet is replaced by a good ration, while in others death occurs irrespective of the change of food. The general symptoms are not definite enough to give any clue as to the degree of injury.

Animals were fed on toxic cotton seed meal until marked illness was apparent, then the diet was changed to the control corn meal mixture. Rabbit 62 ♂ fed for eighteen days on ether-extracted cotton seed meal I lost weight and became ill, did not recover on the corn meal mixture but died on the twenty-first day. On the same deleterious food rabbit 63 ♂ became sick on the twenty-third day. Although the diet was changed death occurred on the twenty-ninth day. Both autopsies revealed intestinal ulcers and hemorrhages. After the rabbits once sickened on the cotton seed meal they never regained their appetites, even though they were transferred to a diet which rabbits normally relish.

Guinea-pig 12 ♂, after sixteen days on cotton seed meal I became sick and weak. The diet was then changed to the corn meal mixture on the seventeenth day but the animal continued to decline; on the twenty-fourth day 1 gram of yeast, a rich source of water-soluble vitamines, was incorporated daily with the food but this had no beneficial effects as death occurred on the twenty-sixth day. Post mortem examinations revealed typical signs of cotton seed meal poisoning. Guinea-pigs 13 ♀, 15 ♀, and 16 ♂ recovered from the harmful effects of a cotton seed meal diet. These results have been discussed elsewhere, see page 374.

Experiments conducted on pigeons gave dissimilar results. Pigeons 13 ♂ and 14 ♀, fed on cotton seed meal II extracted with ether exhibited great weakness on the twelfth day hence the diet was changed to the corn meal mixture plus 1 gram of yeast daily. Neither the yeast nor the diet had a beneficial effect, as both birds died three days later. Pigeon 17 ♀ suffered a similar fate. It became weak and emaciated after eighteen days of feeding cotton seed meal II; 1 gram of yeast was added to the diet but death occurred six days later. Quite to the contrary, pigeon 18 ♀ although very much indisposed after a similar treatment with cotton seed meal, was greatly improved six days later on corn meal mixture alone and after twenty-eight days of the good diet the bird was in splendid condition.

The experience with mice varied. As will be recalled from previous experiments, mice declined on a supplemented diet which contained 50 per cent cotton seed meal II. In some cases, these mice recovered on a comparable diet in which the cotton seed meal was replaced by corn meal mixture, in others they died. But in all cases where dog biscuits were used as a substitute for the deleterious diet, the mice recovered.

INFLUENCE OF COTTON SEED MEAL ON REPRODUCTION

Reports on the influence of feeding cotton seed meal on reproduction are comparatively few in number and varied in character; some animals are affected by the meal, others are not. Barnett (1909), Ward (1915), and others have found that large quantities and long continued feeding of cotton seed meal caused a larger percentage of abortions among breeding cattle and difficulty was experienced in getting animals pregnant. Large amounts of cotton seed meal seem to have no ill effects on pregnant ewes according to Gray and Ridgeway (1910). Investigators disagree in the effect of cotton seed products upon poultry. Richardson and Green (1917b) found in their experiments with rats on cotton seed rations that "normal growth and reproduction do not result from diets containing 50 per cent of cotton seed flour in which there is a lack of butter fat, protein-free milk, or both."

In view of this fact, the present investigator attempted a study on the reproduction of mice when fed upon the supplemented control diet, containing 50 per cent cotton seed meal I, 5 per cent butter fat, 4 per cent inorganic salt mixture and 41 per cent lard. The results lead one to conclude that such a diet is not conducive to reproduction; for out of the six female mice used four died during parturition, and two were able to bear two litters of young but they died shortly after the second litters were born. From the four female mice of the second generation one was able to bear a litter of four young but the mother and young died a few days afterwards. From our limited experience with mice we would conclude that mice do not repro-

duce normally on a cotton seed meal ration which has not proven actively toxic to mice when supplemented with fat soluble vitamins and inorganic salts.

PATHOLOGICAL STUDIES¹⁵

The most characteristic clinical symptoms of cotton seed meal injury of all animals are those of marked loss in body weight followed by emaciation and loss of appetite, weakness and incoordination of muscles as recognized by an unsteady gait, rough hair coat, disturbance in breathing and in some cases impairment of sight. During the last stages of the injury there is an inability to remain on the feet and the animal either lies in a coma or there is a vigorous movement of the limbs as if in an attempt to regain the normal standing position. However, the general symptoms are not definite enough to give any clue as to the degree of injury.

Macroscopic post mortem examinations of animals that have died as the result of cotton seed meal injury do not always reveal a definite apparent lesion. There are variations in the observations made on different animals and even within the same species the gross anatomical changes may be very unlike. However, the more frequent lesions observed in animals are: congestion and hemorrhages in the intestines, congestion of the eliminative organs as the liver and kidneys, oedema of the lungs. In the present investigation the most characteristic findings in the gross post mortem examination of rabbits and guinea-pigs that died from the effects of cotton seed meal feeding was the dilation of the right side of the heart. There was usually congestion of the liver, kidneys, and in many cases the lungs. Splanchnic congestion was frequent, and if the intestine was not hemorrhagic it appeared friable and was easily ruptured. The stomachs of the rabbits were usually filled with cotton seed meal coated with a thick layer of mucus. The stomachs of the guinea-pigs

¹⁵ The authors gratefully acknowledge the assistance of Prof. Milton C. Winternitz of the Department of Pathology and Dr. Frank P. McNamara of the New Haven Hospital in making careful post mortem examinations of the animals used in this investigation.

were generally quite empty, but the cecum was distended with a meal residue in each case. Pigeons and mice revealed no characteristic gross lesions.

Microscopic studies of the internal organs of animals that have died from cotton seed meal injury will be reported at a later period.

SUMMARY AND CONCLUSIONS

Cotton seed *kernels*, alone or mixed with other food stuffs, are unpalatable and fatally toxic to rabbits, guinea-pigs, and pigeons even when fed with molasses and cabbage; and also to albino mice even when the kernels are supplemented with butter fat and inorganic salts.

Rabbits and guinea-pigs decline rapidly on cotton seed *meal*; pigeons and mice decline more slowly, depending upon the source and properties of the meal fed; for different samples of cotton seed meal vary in their immediate effects upon animals.

Cotton seed meal was rendered less deleterious and more palatable to rabbits by treatment with moist heat at high temperatures. Thorough extraction with ether was ineffective in detoxicating cotton seed meal.

The decline of animals feeding on cotton seed meal, resulting in the so-called "cotton seed meal injury," is not attributable entirely to inanition. This was demonstrated by determining the food intake of each animal and by conducting control experiments; the latter, in which animals received daily the same quantity of an adequate control diet as that which the cotton seed meal victims consumed, show that death is not the result of starvation but due to some other factor.

Cotton seed meal injury is not due to a lack of water-soluble vitamine; firstly, because all cotton seed diets were fed to rabbits, guinea-pigs, and pigeons along with a large quantity of cabbage which is well known to contain sufficient vitamins; secondly, because mice survived six months in good conditions on a diet in which the only source of water-soluble vitamine was cotton seed meal; thirdly, because toxic cotton seed meal contains enough vitamine to completely relieve pigeons suffering from

polyneuritis in two to five days; fourthly, because yeast, which is very rich in water-soluble vitamine, incorporated in a toxic cotton seed meal diet is ineffective in warding off cotton seed meal injury.

Some animals are able to recover from the ill effects of cotton seed diets when the deleterious food is replaced by an adequate one, others are not.

Normal reproduction of albino mice does not take place on a food mixture containing 50 per cent cotton seed meal, 5 per cent butter fat, 4 per cent inorganic salt mixture—a diet which one would regard as adequate; the second generation tends to be very weak and unable to reproduce in the normal way.

The most characteristic clinical symptoms of cotton seed meal injury in rabbits, guinea-pigs, pigeons, and albino mice are emaciation, loss of appetite, weakness, rough hair and unkempt appearance, disturbance in breathing, finally coma and perhaps paralysis.

Macroscopic post mortem examination reveals dilation of the right side of the heart, usually congestion of the liver, kidneys, and in many cases, the lungs. Splanchnic congestion was frequent, and if the intestines were not hemorrhagic they often appeared friable and easily ruptured.

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THE INFLUENCE OF INTRAVENOUS INJECTIONS OF ACACIA-GLUCOSE SOLUTIONS ON URINE EXCRE- TION AND BLOOD VOLUME IN RABBITS

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The possibility of using acacia solutions intravenously in various clinical disorders suggested a series of animal experiments. We were particularly interested to learn how far acacia might be relied upon to keep up the blood volume in spite of a marked loss of water through the kidneys. Knowlton (1) found that both acacia and gelatin in 5 per cent solutions were effective in inhibiting saline diuresis. He believed the decreased secretion to be due to a change in concentration and therefore a change in the osmotic pressure of the blood colloids. The decrease in secretion is explained by the hypothesis put forth by Starling (2) in which the secretion of urine is regarded as a filtration process due to the difference between the capillary blood pressure and the osmotic pressure of the blood. Spiro (3) found in his study of the diuretic action of colloids, that their injection had but little effect on the output of urine in animals that had been starved from thirty-six to forty-eight hours prior to experimentation. In animals fed on a diet containing a high percentage of water, the injection of a 3 per cent gum solution caused an increase in urinary secretion. Moutard-Martin and Richet (4), in their experiments with sugar diuresis, observed that the sugar polyuria could be arrested by the injection of gum solution. They noted further that the decrease in urinary secretion following the injection of gum solution took place in spite of an increased blood pressure, whereas in the sugar polyuria the blood pressure was not increased, and might even be less than normal. Kruse (5)

states that urine secretion is diminished and at times nearly suppressed after acacia injections and he regards this as a factor in the maintenance of blood volume by acacia. He further states that the blood volume is well maintained, partly at the expense of urine secretion.

Our injections were given into the ear vein of rabbits. The experiment was so arranged that a given animal received on a given date an intravenous injection of glucose at the rate of 7 grams per kilogram of body weight per hour. After several days the same animal received a solution of glucose containing 6 per cent acacia, the glucose being given at the same rate as before. The urine was collected for the duration of the experiment—the bladder being emptied by expressing the urine at the beginning, at the end and one hour after the injection. Sometimes there was a variation in weights of the animals between the experiments, but it is doubtful whether this was enough to be of much influence on the results. We used glucose at this rate of injection for its powerful diuretic effect. The injections were given by a Woodyatt (6) motor-driven syringe.

In some preliminary experiments, comprising a series of eight rabbits, the injections were given for a period of one hour. This was found to be too short a time for the establishment of a marked diuresis in every case, so the time of injection was increased to one and one-half hours. The results of the latter experiments are given in table 1. The amounts of fluid injected and excreted, the difference between these amounts and the excretion per kilogram per hour is given in separate columns. In addition, hemoglobin determinations, made according to the Palmer (7) method are recorded. These readings were made at the beginning, at the end, and one hour after the close of the experiment. We were interested in the changes of hemoglobin in a given experiment, not in absolute values. The same standard was used for each experiment, but as the standards were kept for a week or more they may have changed, a possibility which has been pointed out by Cohen and Smith (8). Neither the injection of glucose nor of acacia-glucose leads to a withdrawal of red blood cells from the circulation as far as is known. The hemoglobin deter-

minations indicate, therefore, the changes in the blood volume. The last column of each table shows this percentage change. Our acacia solutions were not prepared with 0.9 per cent sodium chloride in place of water as Bayliss (9) demands, in order to make them isotonic with the red blood cells, but the glucose con-

TABLE 1

Glucose

NUM- BER	WEIGHT	INJECTED IN 1½ HOURS	EXCRET- ED IN 1½ HOURS	EXCRET- ED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR (2½ HOURS)	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME IN 2½ HOURS
							Begin- ning of injec- tion	End of injec- tion	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.0	58	110	135	77	26	51	69	79	-3.5
2	1.7	56	150	150	94	35	83	63	100	-17
3	1.9	60	200	230	270	48	76	63	86	-11
4	2.02	56	200	200	144	40	86	100	111	-24
5	2.0	64	145	160	96	32	83	74	100	-17
6	2.07	64	150	150	86	31	83	86	96	-15
7	2.23	72	180	250	178	45	85	83	100	-15
8	2.46	74	180	220	146	36	71	78	83	-14
9	2.78	84	240	320	236	46	63	86	100	-33

Six per cent acacia glucose

1	1.87	56		90	34	20	75	50	72	+4
2	1.75	45		61	16	14	61	56	62	-2
3	1.8	68		210	142	46	75	71	65	+15
4	1.89	55		170	115	36	69	59	62	-3
4	1.95	68		118	50	24	71	48	71	0
5	1.75	45		100	55	23	83	82	83	0
6	2.19	53		180	127	32	71	71	72	-2
7	2.165	52		190	138	35	72	64	70	+2.5
8	2.14	60		150	90	28	64	61	63	+2
9	2.67	61		?	—	—	77	78	78	-1

centrations, between 30 and 40 per cent, were such as to make our solutions markedly hypertonic.

A rule, the urine excretion from glucose alone, in these nine animals exceeded that following acacia-glucose. The preliminary series of eight animals referred to above, also showed, on the whole, some diminution in the urine output with acacia-glucose when compared with the glucose alone. The changes in the

blood volume as shown by the hemoglobin readings at the end of the glucose experiments were variable: in some the blood volume was increased, in others there was a decrease. The percentage increase or decrease of the blood volume was calculated, using as a basis a blood volume equal to 5.5 per cent¹ of the body weight. At the end of the injection the blood volume varied between a concentration of 26 per cent and a dilution of 32 per cent. But, without a single exception, the readings one hour after the injection showed a decided decrease in the blood volume. This concentration varied between 11 and 35 per cent. It is therefore clear that when glucose is given alone the withdrawal of fluids leads to a diminution of the blood volume.

With the injection of 6 per cent acacia and glucose into these animals, the urine output was more or less diminished in all but one compared with the urine output of the same animals with glucose injection. The hemoglobin determinations showed a transitory dilution of the blood in about one-half the cases—this with one exception had disappeared almost entirely one hour later, the final readings being almost identical with the initial readings. The increase in blood volume amounted in two cases close to 50 per cent; in the others it varied between zero and 12 per cent. One hour later it was 15 per cent in one case, in the others it varied but little from the initial. This is in marked contrast to the concentration observed in the glucose experiments. This happened in spite of a quite marked diuresis as may be seen in the table.

The nine glucose animals of table 1 serve also as controls for the experiments recorded in tables 2 and 3.

Table 2 gives the results of 12 per cent acacia-glucose injections in six rabbits. The amount of urine excreted is variable—in animal no. 2 there was none at all. In nos. 4 and 5 the amount was great. There is no very marked difference in the output of urine when compared with that of the animals receiving 6 per cent acacia-glucose. In these experiments the urinary output was only recorded during the period of injection. The blood vol-

¹ Meek and Gasser (10) give the blood volume of the rabbit at 5.44 per cent of the body weight.

ume is much more uniformly increased than in the other series and more persistently so. The blood volume had increased at the end of the injection from 8 to 62 per cent; one hour later it had slightly decreased in one instance, in another it had practically returned to the starting point, in the rest it had increased from 18 to 50 per cent. In no. 5 with a high urine excretion, there was a slight concentration one hour after close of the experiment and but little more on the following day (twenty hours later). In animals nos. 4 and 6 there was a marked dilution which persisted, though to a less degree on the following day. In no. 2 no urine was voided nor could any be expressed. The result of this experiment is puzzling. We included it in our

TABLE 2
Twelve per cent acacia-glucose

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.4	72	90	18	25	66		50	+32
2	2.17	62	0			56	50	57	-1
3	1.9	60	90	30	32	64	50	54	+18
4	2.17	81	220	139	53	68	42	50	+36
5	2.62	78	270	192	69	69	64	71	-3.5
6	1.71	52	140	88	55	75	51	50	+50

series because we could find no justification for not doing so. It is to be noted that only one animal excreted urine within the next twenty-four hours.

Table 3 gives the results obtained when 3 per cent acacia-glucose is injected. The urinary excretion is not inhibited, in fact the output per kilogram per hour at the end of injection exceeds even that of the control animals in which glucose alone was used. The blood volume, in spite of marked diuresis, remains fairly constant. This is also in marked contrast to the concentration seen when glucose alone is used. At the end of the injection the blood volume was definitely increased twice (10 and 24 per cent). In the other four cases there was little change. After one hour

it was increased 6 per cent in one case, in the remainder it varied but slightly from the initial volume.

It must be stated that no changes indicative of kidney lesions were found either before or after the experiments, but with two exceptions. No. 2 of table 1 showed hyalin casts after 6 per cent acacia-glucose and in no. 7 of table 4 albumen was found after 3 per cent acacia saline. Neither did the animals show any marked clinical symptoms during the injection nor afterwards. At the end of the glucose experiment in no. 3 of table 1 there were some respiratory changes. All animals were quiet after injections, food being refused until the following day. All lived in apparently good health for several weeks. We tried to deter-

TABLE 3
Three per cent acacia-glucose

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.24	67	210	143	62	82	66	81	+1
2	1.86	54	135	81	51	58	52	56	+3
3	1.88	57	160	103	57	71	71	76	-4
4	1.39	42	165	123	79	62	63	63	-3
5	2.27	69	185	116	54	68	70	71	-4
6	2.03	60	180	120	59	72	71	68	+6

mine whether or not acacia was excreted in the urine by the furfural phloroglucin method. The large and varying amounts of pentosans present in normal rabbits' urine made this impossible.

The simultaneous injection of 6 or 12 per cent acacia and glucose may exercise an inhibitory effect on the glucose diuresis. This effect seems to be variable and not constant. Acacia given in a 3 per cent solution cannot be said to have exercised any inhibitory effect on the glucose diuresis. Indeed it is questionable whether it did not enhance somewhat the diuretic effect of the glucose. An idea of the degree of diuresis obtained with glucose in our experiments may be gained from the fact that the amount of urine excreted over and above the amount of solution injected

approached or even exceeded the total calculated blood volume of the animal. In some of the glucose acacia experiments the same holds true. But while the glucose acacia experiments are followed by a marked concentration of the blood volume, the acacia-glucose injections are not. In spite of a very pronounced diuresis the blood volume is maintained. In all but two cases (table 1, no. 9 and table 2, no. 2) the amount of urine exceeded the amount of fluid injected. In most instances this excess was considerable, and no doubt much greater than the amount of urine which would have been produced spontaneously. Fluid must enter the vessels and is held therein in spite of the diuresis.

TABLE 4
Three per cent acacia in normal saline

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.23	61	36	-25	6.4	56	50	42	+30
2	2.9	80	40	-40	5.2	57	54	50	+14
3	1.8	50	64	+14	14.0	100	83	100	0
4	1.7	48	70	+22	16.0	93	83	86	+7
5	1.56	44	5	-39	1.2	116	85	88	+32
6	3.2	90	94	+4	12.0	72	63	64	+12
7	2.6	74	30	-44	4.6	78	69	72	+8
8	2.46	69	65	-4	10.0	83	71	78	+6

These results support the supposition of Gasser and Erlanger (11) that glucose brings fluid into the circulation and is held there by the acacia. The possibility that the 3 per cent acacia favored diuresis has been noted. Indeed, Spiro, it will be remembered, claimed a diuretic effect of 3 per cent gum solution in animals having received a diet rich in water. In his experiments the gum was dissolved in saline solution, but the injections were given at a rate much exceeding ours.

Our results with 3 per cent acacia in physiological salt solution obtained in eight animals are recorded in table 4. We injected about half of the calculated blood volume within one and one-half hours. In another, smaller series of animals represented by table

5, physiological salt solution alone was given at the same rate. All the animals were on a diet rich in water. In only three of the eight rabbits receiving the acacia did the output of urine exceed the amount of fluid injected, and in only one of the three animals receiving saline alone. Even with this small number of controls it can be said that 3 per cent acacia has no very definite diuretic effect under the conditions of our experiments.

It is impossible to bring the changes of the blood volume in our experiments in direct relation to the renal activity. A great deal of stress has been laid on the importance of osmosis for the maintenance or increase of blood volume by acacia. So Gasser, Erlanger and Meek (12) state that the injection of acacia greatly reduced the transudation of plasma which occurs in traumatic

TABLE 5
Normal saline

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.82	80	30	-50	4.2	96	79	78	+22
2	2.37	67	35	-32	6.0	83	75	77	+6
3	2.4	68	70	+2	11.6	65	63	55	+18

shock. This is one of the main factors in the decrease of the blood volume. The mechanism of its action is seen in the antagonism to filtration by the resulting increase in the osmotic pressure of the plasma colloids. Nevertheless these authors remark that after the injection of 4 cc. per kilogram 25 per cent acacia solution in the dog the maximal actual expansion of the blood never reaches anything like the theoretical calculated from the osmotic pressure of such a solution. This point, they say, is of considerable interest as it minimizes the importance of what would seem to be one of the most obvious explanations of the protective action of acacia, namely, its increase in volume by the attraction of water. Some of our results make us also hesitate to stress too much the importance of the osmotic explanation of the maintenance of blood pressure by acacia. We calculated the

osmotic pressure of the serum of our animals one hour after the end of the injection from the changes in blood volume, adding to it the osmotic pressure derived from the amount of acacia injected. We utilized for this purpose the figures of Gasser, Erlanger and Meek (11) who give the osmotic pressure of dog serum (measured with the aid of a colloidin membrane) as 16.4 mm. Hg., and that of a 7 per cent acacia solution as 22 mm. Hg. Comparing the calculated osmotic pressure in mm. Hg with the increase or decrease in blood volume, no relationship was discernible. True, this calculation of the osmotic pressure can only be done very roughly. It was assumed that neither protein nor acacia leave the circulation. We are aware that the latter is really not the case as Meek and Gasser (10) pointed out. We realize that the conditions of these experiments do not entitle us to any definite opinion. All we can say is that the results make one hesitate to accept the osmotic pressure as the all-important factor in the maintenance of the blood volume by acacia. This doubt is strengthened by the experiments with 3 per cent acacia and saline and with saline alone. In the latter the dilution of the blood cannot be dependent in any way on the osmotic pressure.

CONCLUSIONS

Acacia is capable of maintaining the blood volume in spite of a very marked glucose diuresis. Injecting acacia-glucose mixture wherein the glucose is present in concentrations of 30 to 40 per cent in quantities of about one-half of the rabbit's blood volume in one and one-half hours a decrease of the blood volume is practically prevented by the presence of 3 per cent acacia in the injection fluid. Glucose injections without acacia produce uniformly a diminution of the blood volume.

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THE ANESTHETIC AND CONVULSANT EFFECTS OF GASOLINE VAPOR

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The symptoms and effects of inhalation of gasoline vapor and other petroleum products have been quite widely noted in industry. Accidental deaths in men entering large tanks or reservoirs recently emptied of gasoline sometimes occur. Thus on the clinical side the facts are fairly well established. There seem however to have been heretofore no experimental investigations of this subject.

Following the loss of the life of one of the members of a rescue crew of the Bureau of Mines who entered a gasoline storage tank while wearing a half-hour type of oxygen apparatus, some experiments were recently made by Mr. A. C. Fieldner, S. H. Katz, and S. P. Kenney at the Bureau of Mines Experiment Station at Pittsburgh. They found that even when mixed with a high concentration of oxygen in a breathing apparatus, 2 to 2.5 per cent of gasoline vapor renders a man dizzy and soon becomes intolerable.

There is a tendency among persons unacquainted with the potency of carbon monoxide as an asphyxiant to think that gasoline vapor—that is volatilized unburned gasoline—is the principal or at least a considerable factor in the toxicity of exhaust gas from engines. Such however is not the case.

In the course of investigations carried out here by Professor Henderson and a staff, of which the writer was a member, under the United States Bureau of Mines for the Commissions of New York and New Jersey charged with the construction of the proposed vehicular tunnels under the Hudson River, the whole

question of the physiological effects of exhaust gas has been investigated. The experiments to be here reported were performed in order to determine the possible part which gasoline vapor might play. They show that although gasoline vapor has an intoxicating effect, the toxicity of a given amount of this vapor is vastly less than that of the carbon monoxide produced by its (incomplete) combustion in an engine.

The following experiments were carried out on dogs. They were placed in a glass chamber of approximately 11 cubic feet. A stream of air of 20 liters per minute was blown through a flask partly filled with gasoline, and on through a short piece of glass tubing into the chamber. The flask stood on an electrically heated plate and the dosage of gasoline was controlled by the amount of heat.

Air samples were drawn from the chamber at intervals and analyzed for their content of gasoline vapor by means of the Henderson-Orsat apparatus. Alboline, a heavy petroleum oil sold for medicinal purposes, was used for the absorbent.

In successive experiments the principal brands of gasoline sold locally were used. Its volatility proved to be surprisingly low. In fact, not much above the fatal value of vapor concentration. We are informed, however, that measurements at the Bureau of Mines Experiment Station at Pittsburgh give for the gasoline generally on the market a volatility two or three times as great as that used in these experiments.

The physiological effects in all of our experiments at various concentrations of vapor were virtually identical. The tabulated experiments are typical.

The data of these experiments are plotted in figure 1. The close agreement between the concentrations at which the various symptom phases occurred is noteworthy. In general gasoline vapor has an anesthetic action somewhat like that of ethyl ether but with marked convulsant effects due doubtless to irritation of the cerebral cortex. Thus the stage of excitement, common in ether anesthesia, is marked by convulsions under gasoline vapor. The stage of full anesthesia, between consciousness and death, is very narrow.

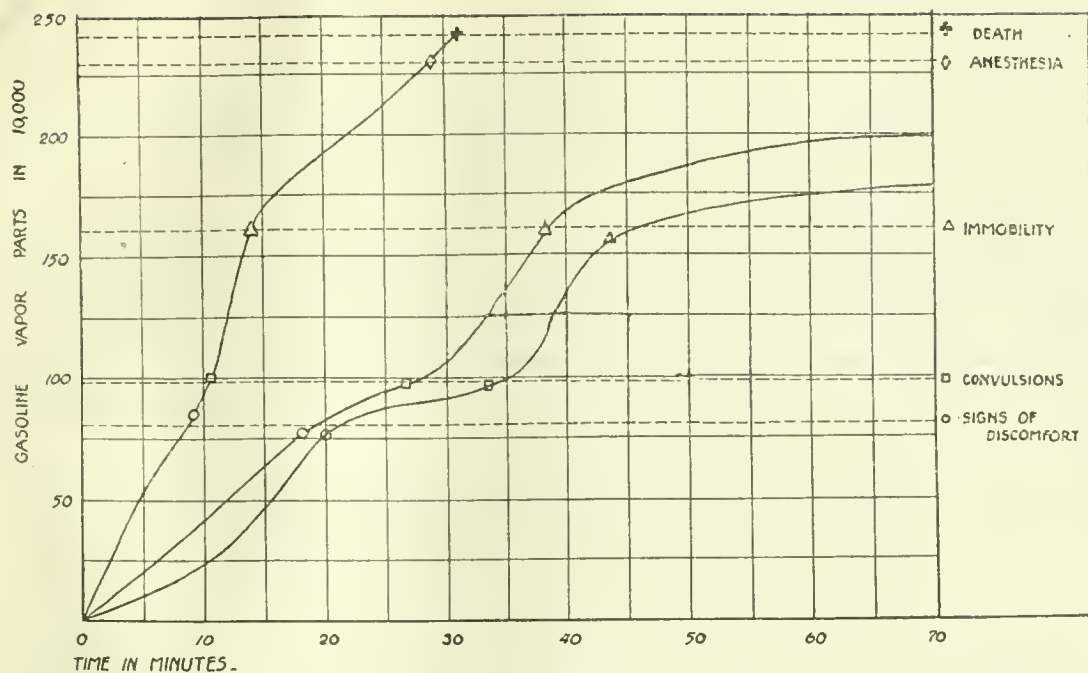


FIG. 1

Experiment 1. Dog, male, 12 kilos. "Standard" gasoline

TIME	CONCENTRATION OF VAPOR	NOTES
<i>minutes</i>	<i>parts in 10,000</i>	
0	0	Vaporizations started
12	32	No symptoms
20	79	Slightly depressed, licks nose
28	89	Slight general tremor, restless
31		Fell. Violent clonic spasm. Epileptiform convulsions
36	100	Tried to stand, but falls repeatedly. General muscular tremor. Salivating
42	156	Animal becoming weak, unable to stand. Fairly quiet though conscious. Chamber cloudy with condensing vapor
65	176	Conscious. Quiet
73	175	Animal taken out of chamber, and laid on the floor

After removal from chamber

2		Unable to stand although making violent efforts
5		Rolls on the floor. Clonic muscular spasms
9		Raised body on four legs. Limbs rigid
12		Stands very unsteadily with legs wide apart
13		Fell on attempting to walk
16		Attention attracted by calling. Walks but falls repeatedly
22		Walks fairly well, slight general muscle tremor
Next day		Slightly depressed. No appetite. Drinks water
Second day		Apparently perfectly normal

Experiment 2. Dog, male, 11 kilos. "Socony" gasoline

TIME	CONCENTRATION OF VAPOR	NOTES
<i>minutes</i>	<i>parts in 10,000</i>	
0	0	Vaporization started
18	76	Animal slightly restless
30	102	Violent spasms, unable to stand. Head retracted, general rigidity
38	162	Almost continuous epileptiform convulsions
46	184	More quiet. Breathing shallow. Conscious
62	196	Conscious, quiet. Chamber temperature 22°C.
64		Animal taken out of chamber and laid on floor
After removal from chamber		
4		Violent epileptiform convulsions for 62 seconds, during which respiration stopped
8		Continued convulsions
12		Convulsions less violent
20		Attempted to stand
25		Able to stand but falls on attempting to walk
30		Walks but staggers
Next day		Depressed. No appetite
Second day		Appears normal except for slight inflammation of one eye

Experiment 3. Dog, male, 16 kilos. "Good Gulf" gasoline

TIME	CONCENTRATION OF VAPOR	NOTES
<i>minutes</i>	<i>parts in 10,000</i>	
0	0	Vaporization started.
5	52	No symptoms
10	96	Restless, licking nose
13	160	Slight general convulsion, unable to stand
19		Violent epileptiform convulsions
22	200	Muscular rigidity, very shallow breathing
31	235	Unconscious, shallow breathing. Temperature of chamber 26°C.
37	243	Feeble spasms
39		Dog dead

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V. FURTHER STUDIES ON THE ANTAGONISTIC ACTION OF EPINEPHRIN TO CERTAIN DRUGS UPON THE TONUS AND TONUS WAVES IN THE TERRAPIN AURICLES

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The effect that nicotine has upon the contracting auricles in situ was first studied by Fano and Sciolla (1) and later reviewed by Bottazzi (2). The former experimenters report that a 1 per cent solution of nicotine dropped upon the auricles does not alter the general tonus but causes the tonus waves to disappear and the strength of the contractions to be increased. A 0.5 per cent solution of nicotine was found by Bottazzi to decrease the tone of the muscle. Barry (3) noted ventricular muscle to be more susceptible to the influence of nicotine than auricular muscle.

While working with potassium chloride and the auricles the author used nicotine in an attempt to counteract the effect that potassium chloride has upon the tonus waves and tonus; but was surprised to find that it increases rather than decreases the tonus as found by the above authors. Consequently the present problems were undertaken (1) to determine the effect of nicotine upon excised auricles; (2), if nicotine produces an increase in tonus what would be the effect of epinephrin upon it. (3) The effect of epinephrin upon the increased tonus produced by the actions of digitalis and of barium chloride was also noted.

THE METHOD

The method employed in this work was the same as that used in a previous work and described in detail elsewhere (4). The animals were terrapin of the species *Chrysemys elegans*.

RESULTS

1. *Nicotine.* The results with nicotine varied according to the strength of the solution used. An increase in tonus and tonus waves was obtained with solutions of 0.2 to 1 per cent strength. With weaker solutions, 0.01 to 0.05 per cent, only a decrease in tonus and in some cases a disappearance of the tonus waves was observed.

Figure 1 is a typical curve in which a strong solution of nicotine was used. At (1) sufficient nicotine was added to the Ringer's solution in which the auricles were immersed, to make a

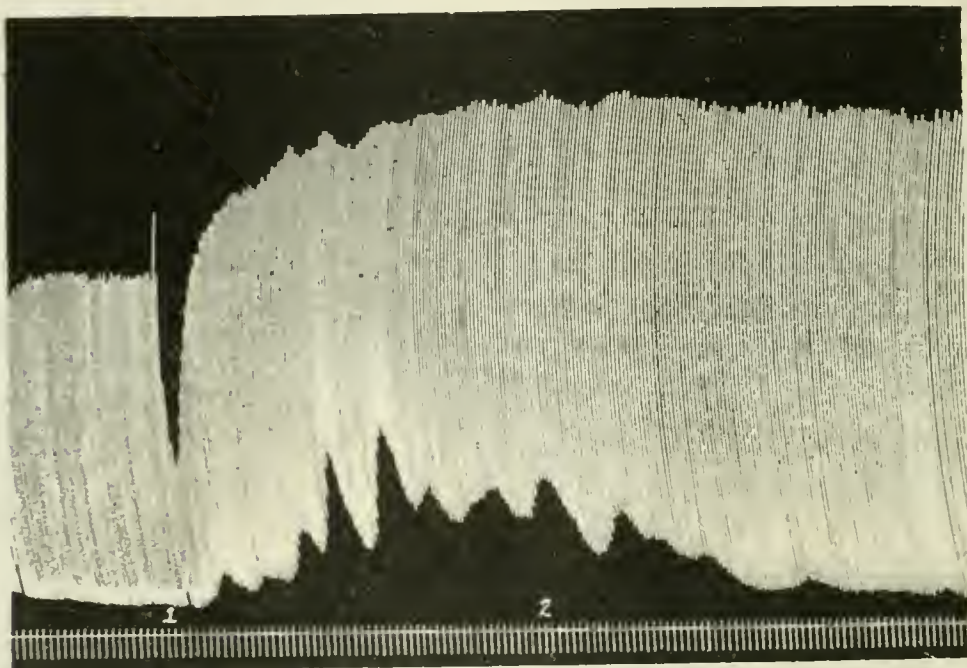


FIG. 1. In this and the following records the upper curve is that of the contracting auricles and the lower line that of the time interval in 5 seconds. Upstroke systole, downstroke diastole. 1, Nicotine 0.5 per cent; 2, Ringer's solution.

0.5 per cent solution. The height of the contractions, at first, quickly decreased, but later the general tonus and tonus waves and the height of contractions increased. At (2) the nicotine solution was replaced by Ringer's solution. The tonus waves disappeared and the general tonus decreased whereas the height of contractions were markedly increased. These results with strong solutions of nicotine do not substantiate those of Fano's

and Sciolla's and Bottazzi's upon the intact auricles. This difference in our results is probably due to the difference in the strength of nicotine used as can be seen in figure 2. In this figure, at the arrow, a sufficient quantity of nicotine was added to the Ringer's solution to make a 0.012 per cent solution. After a temporary slowing and a decrease in the strength of contrac-

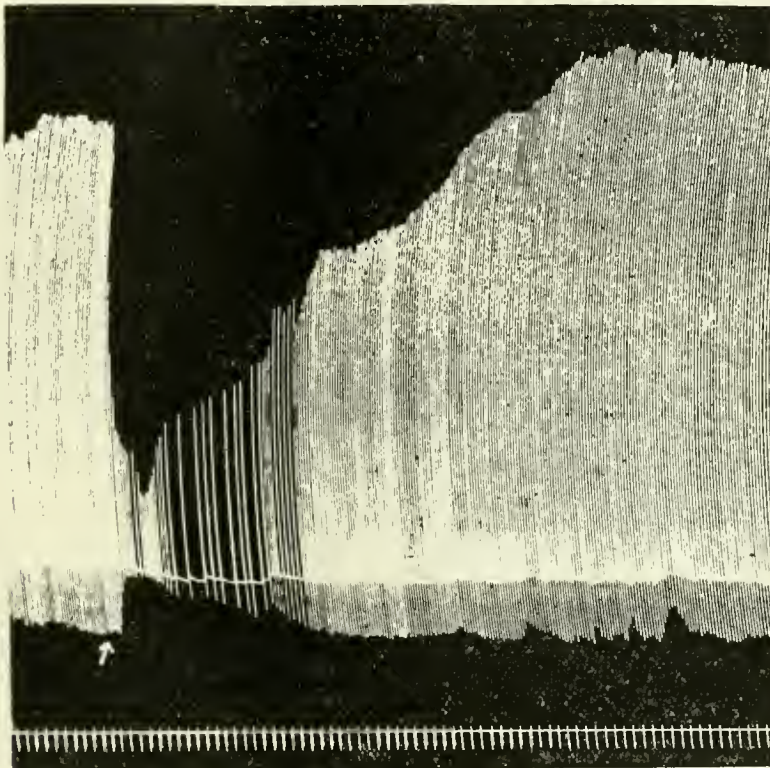


FIG. 2. At the arrow nicotine was added to make a 0.012 per cent solution.

tions there was an increase in the force of contraction with no apparent change in tonus. My results with weak solutions of nicotine confirm those of the previous workers.

Nicotine and epinephrin. In figure 3 (at 1) potassium chloride was added to the Ringer's solution to make a 0.23 per cent solution. The potassium chloride increased the rate of contractions, the tonus and the tonus waves. At (2) nicotine was added to the solution to make a 0.2 per cent solution which further increased the tonus of the muscle. Without changing the fluid (at 3) adrenalin chloride was added to make a 1:20,000 solution.

Almost at once the tonus decreased and the tonus waves disappeared. It was observed that fairly strong solutions of adrenalin were necessary after nicotine had increased the tonus and tonus waves, to make them disappear. This can be explained partially by the rapid oxidation of adrenalin if the color of the solution may be taken as an index of adrenalin destruction by the nicotine. In these cases where the nicotine solution was strong the fluid very quickly (within a few minutes) became pink in color, indicating a change in the adrenalin chloride. The rapidity at

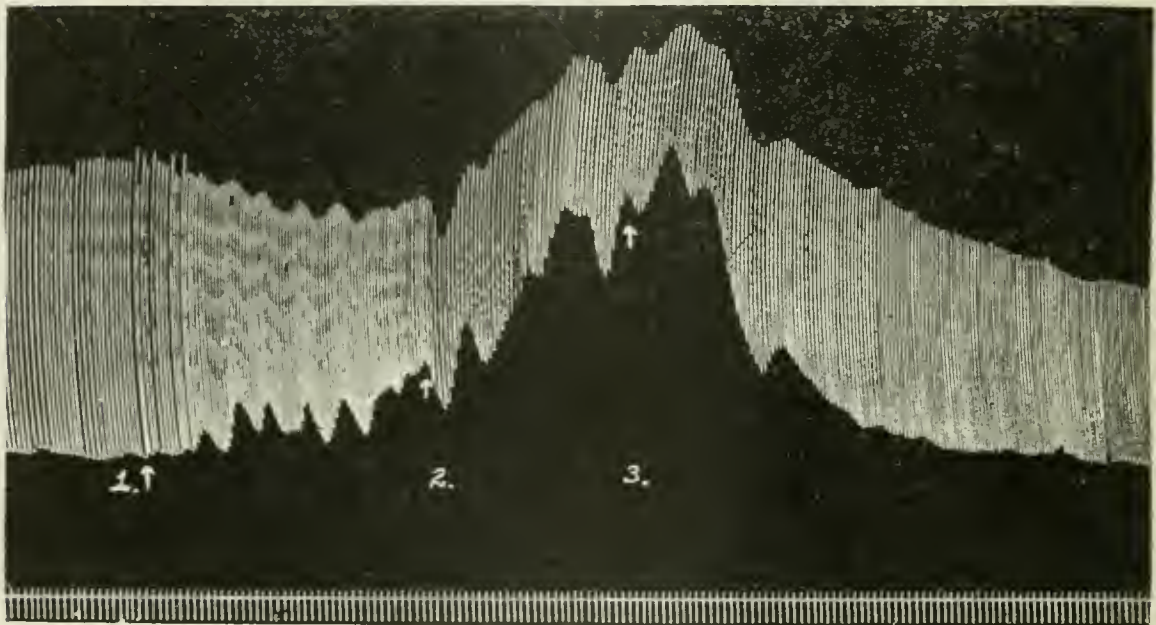


FIG. 3. 1, Potassium chloride 0.23 per cent; 2, nicotine 0.2 per cent; 3, adrenalin chloride 1:20,000.

which the color appeared depended upon the concentration of the nicotine and partly upon the concentration of adrenalin chloride.

2. *Digitalis*. A concentrated solution of digitaline dropped upon the intact beating auricles was found by Fano and Sciolla (1) to decrease the height of contractions and tonus waves but to increase the general tonus of the organ. This increased general tonus was also observed by Bottazzi (2) using the same method and the same experimental animals.

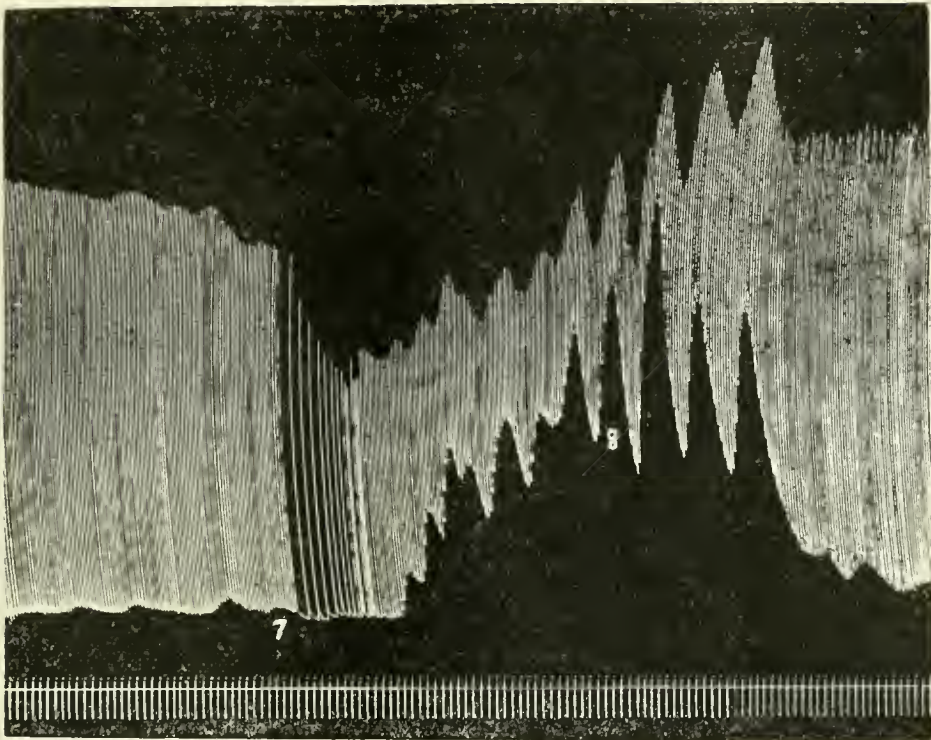


FIG. 4. 7, Tincture of digitalis 3 mils. in 97 mils. of Ringer's fluid; 8, Ringer's fluid.

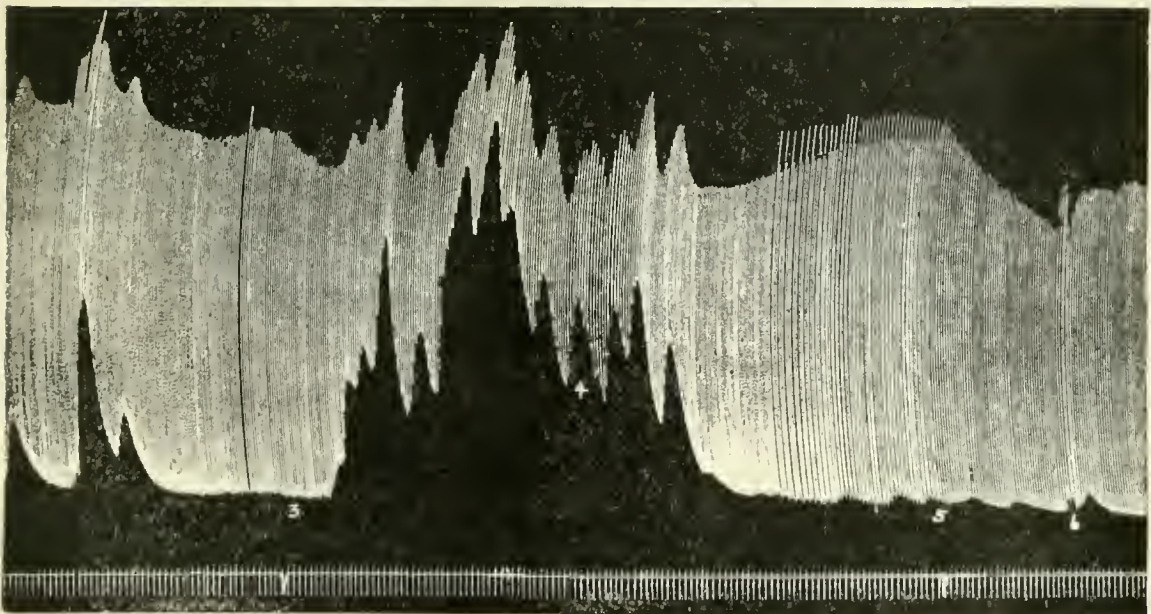


FIG. 5. 3, Tincture of digitalis 4 mils. in 96 mils. of Ringer's; 4, Ringer's fluid; 5, alcohol 3 per cent; 6, Ringer's fluid.

In my experiments, digitalis in 0.3 to 0.5 per cent solutions caused an increase in general tonus and tonus waves with a decrease in the height of muscular contraction. The tincture of digitalis was added directly to the solution in which the muscle was contracting. The effect upon the contracting auricles can



FIG. 6. 1, Tincture of digitalis 4 mils. in 96 mils. of Ringer's fluid; 2, adrenalin chloride 1:33,000.

be seen in figures 4, 5 and 6. In figure 4 (at 7) 3 mils. of tincture of digitalis, fat free, was added to the 97 mils. of Ringer's solution, making a 0.3 per cent solution.¹ A marked increase in the general tonus and tonus waves is the result. At (8) in the same

¹ This calculation is made on the basis of a 10 per cent tincture of digitalis.

figure the digitalis solution was replaced by Ringer's fluid. That the alcohol in the tincture could not have caused the change in the tonus waves can be seen in figure 5. In this figure (at 3) tincture of digitalis was added to the Ringer's solution to make about a 0.4 per cent strength which was replaced by fresh Ringer's (at 4). At (5) alcohol was added to the Ringer's fluid to make

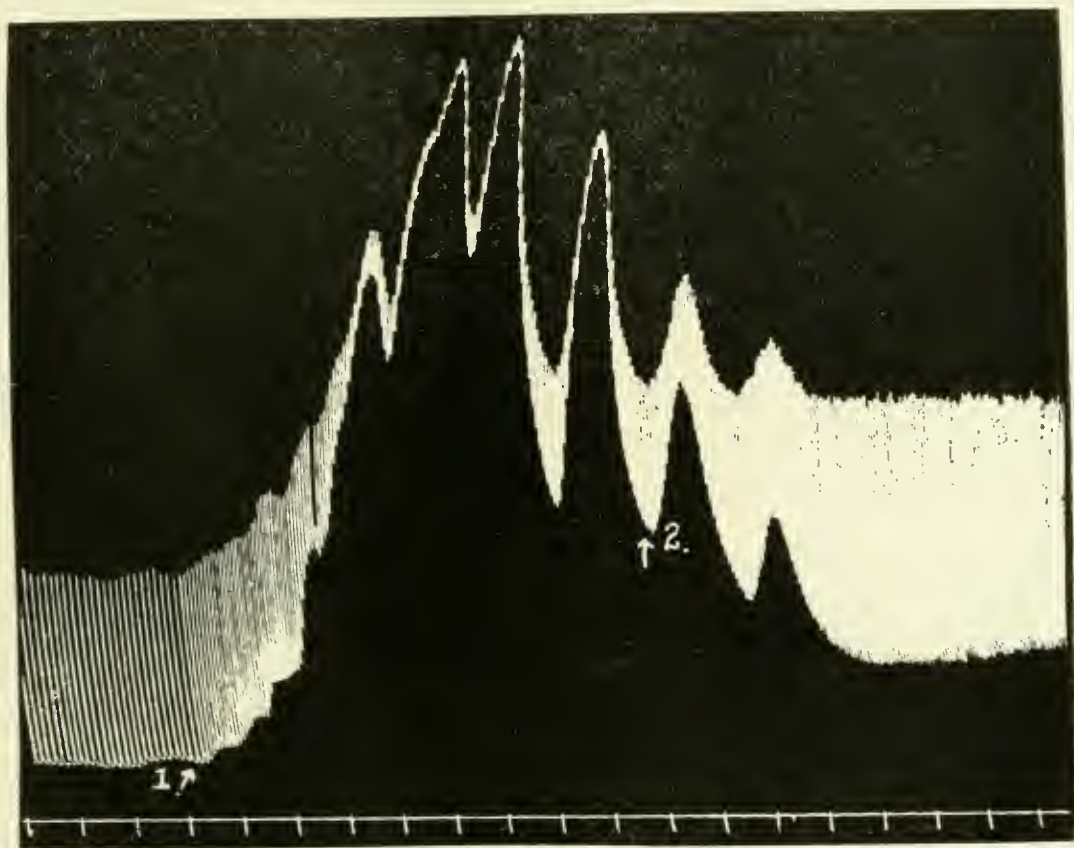


FIG. 7. Upper record the contracting auricles lower record time in 30 seconds. 1, Barium chloride 0.1 per cent; 2, adrenalin chloride 1:50,000.

approximately a 3 per cent solution for which was substituted Ringer's fluid (at 6). The effect of alcohol is entirely different from that of digitalis. In this and in all the other experiments which were performed alcohol did not cause increased tonus nor tonus waves and in my experiments it had no effect upon the tonus waves when they were present.

Digitalis and adrenalin chloride. The effect of adrenalin chloride upon the increased tonus and tonus waves by digitalis can be

seen in figure 6. In this figure (at 1) 4 mils. of the tincture of digitalis was added to the Ringer's fluid in which the muscle was contracting making a 0.4 per cent solution. At (2) without replacing the digitalis by fresh fluid, adrenalin chloride (1:1,000) was added to make approximately a (1:33,000) dilution. The general tonus at once decreased and the tonus waves disappeared, but the contractions increased in strength in the same manner as in a normal muscle.

3. *Barium chloride.* No one as far as I know has studied the effect of barium chloride upon the tonus and tonus waves in the auricles. A number of men noted the action of barium chloride upon the ventricles. Ringer and Sainsbury (5) observed an increased frequency of beat when the frogs ventricle was perfused with a solution containing barium chloride. Poulsson (6) noted a difference in the action of the ventricle of a frog when the barium chloride was applied by dropping the solution upon the heart and when the heart was perfused by it. In the former case the diastolic phase was lengthened, in the latter the heart stopped beating in systole. A decreased responsiveness of the frogs ventricle to calcium, after it had been perfused with a solution containing barium, was noted by Burrige (7). He also noted increased tonus and stoppage of the heart in systole with a 0.3 to 0.5 per cent barium solution. A trace of adrenalin added to the solution containing the barium enabled the heart to double the period of visible activity.

In my experiments barium chloride in all cases produced an increase in the rate of contraction, a decrease in the height of contraction, and an increase in the general tonus and tonus waves. Figure 7 is a typical curve. At (1), 1 mil. of a 10 per cent barium chloride solution was added to the 99 mils. of normal saline in which the auricles were contracting, making a 0.1 per cent solution. A marked increase in the tonus waves, general tonus and rate of contractions occurred at once. At (2) 2 mils. of (1:1,000) adrenalin chloride was added to the barium chloride solution. The tonus waves soon decreased and disappeared and the general tonus decreased some but did not return to the normal level. In the ten experiments performed, in not a single

case was adrenalin chloride effective in lowering the general tonus to the original level and in two cases it was unable to cause the tonus waves to disappear.

This inability of adrenalin chloride to counteract the action of barium chloride is probably due to the fact that the former drug acts upon the sympathetic nerve endings (8) whereas the latter acts upon the muscle directly.

SUMMARY

Nicotine in 0.1 to 1 per cent solution causes an increase in the general tonus and tonus waves. The height of the contractions may be either increased or decreased depending upon the increased general tone.

In small doses of 0.03 to 0.05 per cent solution there is no increase in general tonus or tonus waves but increased height of contractions.

Adrenalin chloride in strong solutions antagonizes the action of nicotine upon the terrapin auricles.

Digitalis in solutions, of from 0.3 to 0.5 per cent causes increased tonus and tonus waves which are antagonized by adrenalin chloride.

Barium chloride caused an increase in rate, general tonus and tonus waves in the terrapin auricles. Adrenalin chloride antagonizes the tonus waves but does not effect the general tonus to the same extent.

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ABSORPTION FROM THE PERITONEAL CAVITY

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Numerous workers have determined the rate of absorption of fluids of varying osmotic pressure from the peritoneal cavity, but less attention has been paid to the question of the rate at which solutions of different salts are absorbed, when they are introduced in isotonic solution.

The experiments described below were undertaken to compare the rates at which salts and fluids were absorbed, when isotonic solutions of different substances were introduced into the peritoneal cavity.

LITERATURE

Orlow (10) showed that serum was slowly absorbed from the peritoneal cavity, and that isotonic solutions of NaCl were absorbed more rapidly.

Hamburger (4) found that both serum and ascitic fluid were absorbed from the peritoneal cavity, and that solutions of various salts were rapidly absorbed; he also found that when an isotonic solution of sugar was introduced into the peritoneal cavity nearly the whole of the sugar was absorbed in an hour, whilst usually only a small amount of the fluid was absorbed.

Cohnheim (2) also determined the absorption of isotonic solutions of sodium chloride and sugar from the peritoneal cavity and obtained results similar to those mentioned above.

Cohnheim concluded that the differences in the rates of absorption of the different solutions could be explained by the differences in the rates of diffusion of the substances investigated.

Adler and Meltzer (1) found that a solution of 0.75 per cent NaCl was absorbed from the peritoneal cavity of a dead rabbit nearly as rapidly as from that of a live one.

Loeb, Fleischer, and Hoyt (9) made an extensive series of experiments upon the conditions governing the rate of absorption of solutions of 0.85 per cent NaCl, from the peritoneal cavity of rabbits and concluded that this rate was increased by any procedure that caused an increase in the osmotic pressure of the blood; they showed, in particular, that the administration of adrenalin, or the ligature of the renal arteries, produced both a rise in the osmotic pressure of the blood, and an increase in the rate of absorption of fluids from the peritoneal cavity.

EXPERIMENTAL RESULTS

The method of experiment that I adopted was to inject 100 cc. of fluid that was isotonic with the blood, at a temperature of 37°C., into the peritoneal cavity of rabbits. The rabbits had received ordinary food up to the time of the experiment.

After an hour the rabbit was killed, and the fluid removed; the depression of the freezing point of the fluid was then measured, and its chemical composition analysed. The detailed results of the experiments are given in the protocols appended to this paper, and a summary of the results obtained is shown in tables 1 and 2.

DISCUSSION OF RESULTS

a. Sources of error

A series of experiments were first made with isotonic solutions of sodium chloride, and the following sources of error were noted:

1. Occasionally the injection needle passed into the colon; in such cases, when the rabbit was killed, the colon was found distended with fluid, and little or no fluid was found in the peritoneal cavity.

2. The size of the rabbit was found to be of importance, for small rabbits only absorbed about two thirds as much fluid, and three quarters as much solute, as large rabbits.

3. The temperature of the inflowing fluid was also of importance, for, if it fell below 30°C., there was a distinct diminution in the rate of absorption.

4. In all of these experiments the rabbits had been fed up to the time of the experiment; I made a few experiments with rabbits that had been starved for twenty-four hours, but was unable to detect any difference in the rate of absorption.

5. Individual idiosyncrasy was by far the most important source of error, for, when every endeavor was made to obtain similar conditions of experiment, still the rate of absorption varied greatly in different rabbits: for instance in the experiments with sodium chloride solutions, when large rabbits were used, the amount of fluid absorbed varied from 15 to 29 per cent, and the amount of salt absorbed from 25 to 47 per cent.

b. Absorption of isotonic solutions of sodium chloride

When 0.95 per cent sodium chloride was introduced into the peritoneal cavity absorption of both fluid and salt occurred, but the salt was absorbed more quickly than the fluid; hence the chloride content of the fluid fell steadily and after an hour the fluid contained between 0.73 and 0.83 per cent NaCl: on the other hand if an isotonic solution of some other substance such as glucose was introduced then chloride was secreted into the fluid, and the chloride content rose, after an hour, to between 0.326 and 0.485 per cent NaCl.

Similar results have been obtained by other workers (Orlow (10), Hamburger (4), Loeb, Fleischer and Hoyt (9)), and apparently a chloride content in the peritoneal fluid corresponding to a content of NaCl between 0.55 and 0.60 per cent is in equilibrium with the tissue fluids, and hence any fluid remaining for long in the peritoneal cavity gradually approximates to this percentage of chloride, whatever its original composition: such fluids however possess an osmotic pressure that produces a depression of the freezing point of -0.590°C. , whilst 0.6 per cent NaCl only causes a depression of 0.37°C. so that over one-third of the osmotic pressure of fluids, that have remained some time in the peritoneal cavity, is due to substances, other than chlorides, that have diffused out from the tissues. Examination of peritoneal fluids showed the presence of small quantities of calcium, and considerable amounts of protein.

The rate of absorption of both fluid and salt per hour was higher during the first hour than in subsequent hours; for instance a comparison of rows I and VIII in table 1 shows that when NaCl solutions were injected into small rabbits, during the first hour the rate of absorption of fluid and salt was 14.8 and 27.5

TABLE 1

The absorption of solutions of sodium chloride from the peritoneal cavity of rabbits. Averages of experiments. In all cases, unless otherwise stated, 100 cc. of fluid, with $\Delta = -0.560^{\circ}\text{C}.$, were introduced intraperitoneally, at a temperature of $37^{\circ}\text{C}.$, and left in for 60 minutes

NUMBER OF SERIES	CONDITIONS OF EXPERIMENT	NUMBER OF EXPERIMENTS PERFORMED	WEIGHT OF RABBITS	PERCENTAGE OF NaCl IN FLUID REMOVED	PERCENTAGE ABSORBED	
					I Of fluid	II Of sodium chloride
			grams			
I	0.95 per cent NaCl in small rabbits.....	8	877	0.81	14.8	27.5
II	0.95 per cent NaCl in large rabbits.....	7	1680	0.793	23.0	35.6
III	0.95 per cent NaCl introduced at $25^{\circ}\text{C}.$	11	1250	0.797	11.2	25.0
IV	0.95 per cent NaCl introduced at $45^{\circ}\text{C}.$	4	1600	0.835	30.0	38.4
V	0.85 per cent NaCl and 0.1 per cent NaF.....	3	1870	0.625	25.3	45.5
VI	0.95 per cent NaCl and two doses of 2.5 mgm. epinine administered intraperitoneally.....	3	1970	0.81	26.7	36.5
VII	0.95 per cent NaCl. Fluid left in for 30 minutes....	9	1240	0.88	11.2	17.8
VIII	0.95 per cent NaCl. Fluid left in for 180 minutes....	3	880	0.735	29.0	44.8
IX	0.95 per cent NaCl. Fluid left in for 45 minutes.....	2	1480	0.836	17.0	27.2

per cent respectively but when the fluid was left in for three hours these rates fell to 9.6 and 14.9 per cent per hour respectively. Similarly, when fluid was left in the peritoneal cavity of a rabbit for an hour, and was then removed and injected into a second animal, then the amount of fluid absorbed by the second animal

was less than the quantity absorbed, when isotonic NaCl was injected; the figures obtained were as follows: isotonic NaCl, 23 per cent of fluid absorbed in one hour and 29 per cent in three hours; fluid recovered from peritoneal cavity, 16 per cent of fluid absorbed in one hour and 15 per cent in three hours.

These last experiments indicate that the slowing down of absorption, observed when fluid was left for three hours in one rabbit, was not due entirely to changes, such as dilution of the blood, induced in the rabbit by absorption of fluid, but that changes undergone by the fluid in the peritoneal cavity influence its rate of absorption.

The diminution in the rate of absorption cannot be explained by alterations in the osmotic pressure, as this remained nearly constant throughout. On the other hand the composition of the fluid changed after an hour's stay in the peritoneal cavity in that the sodium chloride content of the fluid diminished, and its place was taken by a variety of substances entering from the blood, and the result of this exchange must have caused a diminution in the average diffusion rate of the substances dissolved in the peritoneal fluid. This alteration in the rate of diffusion appears to be the most probable cause for the diminution in the rate of absorption. Further evidence for this view will be given later.

The rate of absorption of sodium chloride solutions was influenced by alterations in the temperature of the fluid injected; when this was introduced at a temperature of 45°C. the absorption of fluid and salt was greater, than when the temperature was 37°C., and when this temperature was reduced to 25°C. the absorption of both fluid and salt were much diminished.

The addition of sodium fluoride to isotonic solutions of both sodium chloride and glucose, caused an increased absorption of both fluid and dissolved substance.

These results with temperature and fluoride indicate that vasodilatation of the peritoneal vessels increases the rate of absorption, and that vaso-constriction produces the reverse effect.

The effect of adding epinine and hemisine to solutions was tested, and it was found to increase slightly the absorption of fluid from isotonic solutions of both sodium chloride and glucose, when introduced intraperitoneally, but it did not have any demonstrable effect upon the absorption of the dissolved substance. Loeb, Fleischer and Hoyt, (9) found that adrenalin increased the absorption of fluid from the peritoneal cavity, and they attributed this increase to a rise in the osmotic pressure of the blood.

c. Absorption of isotonic solutions of various substances

The changes observed in the fluids when isotonic solutions of various substances were introduced intraperitoneally are shown in table 2. The percentages of fluid and solute absorbed from the different solutions are shown in table 3. The figures in this table show clearly that the rates of absorption of both fluid and solute, from the solutions of the different substances, vary greatly, and that the different substances form a series, roughly the same as the well known lyotropic series. The absorption of sodium chloride cannot be compared with that of the other substances, owing to the high content of chloride in the tissues, also the figures for magnesium sulphate are only roughly comparable, because enough magnesium was absorbed to kill the animal in less than an hour.

The rate of absorption of the different substances varies directly with their rate of diffusion, and in table 3 the rates of absorption and the rates of diffusion are compared. Since numerous other physical properties vary concurrently with the rate of diffusion, numerous other parallels could be drawn. The danger of accepting analogous variations as evidence of a common cause, is, however, well illustrated by the history of the researches upon the imbibition of salts and water by gelatine. The earlier experiments of Hofmeister (6) upon imbibition by gelatine plates gave results very similar to those I obtained for absorption from the peritoneal cavity, and this suggested that imbibition was perhaps the dominating factor in determining the rate of absorption;

TABLE 2

The absorption of solutions of various salts, etc. from the peritoneal cavity of rabbits. Averages of experiments. In all cases, unless otherwise stated, 100 cc. of fluid were introduced intraperitoneally, at a temperature of 37°C., and left in for 60 minutes

NUMBER OF SERIES	CONDITIONS OF EXPERIMENT	NUMBER OF EXPERIMENTS PERFORMED	WEIGHT OF RABBIT	COMPOSITION OF FLUID REMOVED		PERCENTAGE ABSORBED	
				I Percent NaCl	II Per cent of other salts	I Of fluids	II Of solute
			grams				
I	Fluid taken from the peritoneal cavity of one rabbit and injected into a second one. (When injected the fluid contained 0.76 per cent NaCl: $\Delta = -0.552^{\circ}\text{C}.$).....	5	1510	0.72		16.0	21.9
II	Fluid as in series I, but left in peritoneum for 180 minutes ($\Delta = -0.540^{\circ}\text{C}.$)	3	1420	0.69		15.0	17.8
III	Glucose 5.3 per cent: $\Delta = -0.557^{\circ}\text{C}.$	6	1590	0.387	2.92	-1.0	44.3
IV	Glucose 5.5 per cent: $\Delta = -0.583^{\circ}\text{C}.$ Fluid left in peritoneum for 180 minutes	3	1456	0.488	1.48	-2.0	72.8
V	Glucose, 5.1 per cent: $\Delta = -0.550^{\circ}\text{C}.$: four doses of 1 mgm. epinine given intraperitoneally.....	5	1586	0.341	3.13	13.0	48.4
VI	Glucose 4.9 per cent and NaF 0.1 per cent. $\Delta = -0.596^{\circ}\text{C}.$	3	1524	0.445	1.66	10.0	69.2
VII	Sodium sulphate anhydrous 1.85 per cent. $\Delta = -0.560^{\circ}\text{C}.$	4	1555	0.299	1.02	13.5	52.7
VIII	Sodium nitrate 1.3 per cent. $\Delta = -0.520^{\circ}\text{C}.$	2	1750	0.378	0.443	33.5	77.4
IX	Sodium acetate ($3\text{H}_2\text{O}$) 2.05 per cent. $\Delta = -0.540^{\circ}\text{C}.$	2	1850	0.438	0.885	24.0	67.3
X	Sodium iodide 2.40 per cent. $\Delta = -0.555^{\circ}\text{C}.$	3	1550	0.442	0.801	31.0	76.5

TABLE 2—Concluded

NUMBER OF SERIES	CONDITIONS OF EXPERIMENT	NUMBER OF EXPERIMENTS PERFORMED	WEIGHT OF RABBITSGrams	COMPOSITION OF FLUID REMOVED		PERCENTAGE ABSORBED	
				I Percent NaCl	II Per cent of other salts	I Of fluid	II Of solute
XI	Mixture of Na ₂ HPO ₄ and NaH ₂ PO ₄ (P ₂ O ₅ 1.22 per cent). Δ = -0.620°C.....	4	1500	0.396	0.569 (P ₂ O ₅)	13.5	58.3
XII	Magnesium sulphate (7H ₂ O) 4.82 per cent. Δ = -0.440°C. (the animal died in about 40 minutes).....	1	1620	0.258	1.470 SO ₄ 0.346 Mg	10.0	30.8 SO ₄ 34.0 Mg

TABLE 3

A comparison between the rate of absorption of solute and fluid from different solutions, when they are left in the peritoneal cavity of rabbits for one hour, and the rates of diffusion of the various substances introduced

SOLUTION	PERCENT-AGE OF SOLUTE ABSORBED	PERCENT-AGE OF FLUID ABSORBED	RATE OF DIFFUSION THROUGH ANIMAL MEMBRANE*	DIFFUSION CONSTANT "k"†
Sodium chloride.....	35.6	23.0	1.75	0.90
Sodium nitrate.....	77.4	33.5	1.71	0.83
Sodium iodide.....	76.5	31.0		0.80
Sodium acetate.....	67.3	24.0		0.67
Sodium phosphate (acid and alkaline mixed).....	58.3	13.5		
Sodium sulphate.....	52.7	13.5	1.31	0.49
Magnesium sulphate.....	32.0	10.0		0.34
Glucose.....	44.3	-1.0	1.0	0.25

* Solutions isotonic with blood, rate of diffusion of glucose taken as unity (Hedin (5)).

† Solutions about $\frac{M}{10}$, and at 10° to 12°C. Landolt-Bornstein (7).

but Hofmeister's experiments were all done with concentrated solutions; and later workers (Lenk (8), Ehrenberg (3)) showed that with concentrations between $\frac{M}{5}$ and $\frac{M}{10}$ the rate of imbibition of salt solutions by gelatine form a series which is nearly the reverse of that of Hofmeister.

Since the rate of imbibition of fluids by colloids is in most cases markedly influenced by the hydrogen ion concentration, I tried the effect of varying this in the case of the phosphate solutions. By mixing isotonic solutions of acid and alkaline sodium phosphate, I obtained acid, alkaline and neutral solutions, at the end of the experiment the fluids were still acid, alkaline or neutral respectively, but the rate of absorption was the same in all three cases.

The rate of absorption of salts from the peritoneal cavity appears therefore to depend largely upon the rate of diffusion of the salt, but imbibition does not appear to be a dominating factor in determining the rate of absorption.

d. The absorption of fluids from the peritoneal cavity

The figures given in table 3 show that the amount of fluid absorbed, from the different solutions examined, varies directly as the diffusion constant of the solute, but the case of glucose is remarkable, for no fluid at all was absorbed in one hour from glucose solutions, although 44 per cent of the glucose was absorbed. Moreover when solution of glucose was left in the peritoneal cavity for three hours, still none of the fluid was absorbed, although 73 per cent of the glucose was absorbed.

These variations in the rate of absorption of fluid from the different solutions, and also the non-absorption of fluid from the solutions of glucose, can however be explained by the ordinary laws of diffusion.

Since the various salts were all freely absorbed, but the rate at which they were absorbed varied according to their rate of diffusion, the peritoneum may be assumed to act as a non-selective membrane allowing free diffusion to all the substances investigated. All of the solutions studied, although isotonic with

the blood, were composed of substances not present in the blood, except the sodium chloride solution, and this contained a considerably higher percentage of sodium chloride than does the blood; hence diffusion produced in all cases a rapid exchange of diffusible constituents between the blood and the peritoneal fluid through the peritoneum.

But supposing the substance in the peritoneal fluid has a higher diffusion constant than the average diffusion constant of the substances which produce the osmotic pressure of the blood, then more salts will leave the peritoneal fluid than enter it, and hence its osmotic pressure will tend to fall, and this will cause an absorption of fluid. On the other hand, if the substance in the peritoneal fluid has a diffusion constant lower than the average diffusion constant of the blood substances, then the reverse will happen, the osmotic pressure of the peritoneal fluid will tend to rise, and fluid will enter the peritoneal cavity.

The variations observed in the osmotic pressures of fluids when left in the peritoneal cavity, and the differences observed in the rates of absorption of solute and fluid from the different solutions all agree very well with the supposition that the chief factor determining absorption was the difference between the rate of diffusion of the substance in the peritoneal fluid, and the rate of diffusion of the blood substances. This hypothesis will not of course account for the absorption of serum or ascitic fluid from the peritoneal cavity, this absorption must be produced by other factors, but since these factors did not cause any absorption of glucose solutions in three hours, they cannot have had much influence on the quantities of fluid absorbed in the experiments under discussion.

The percentages of fluid absorbed from the different solutions when left for one hour in the peritoneal cavity, are shown in table 3 and it will be seen that the amount of fluid absorbed is proportional to the amount of salt absorbed, and also to the rate of diffusion of the salt; this is in itself strong evidence that the absorption of fluid is dependent upon the rate of diffusion of the salt. The only exception is sodium chloride and this cannot be directly compared with the other solutions, because of the high content of chloride in the blood.

The changes in osmotic pressure undergone by the various solutions during their stay in the peritoneal cavity are shown in table 4. These figures show that no well marked changes occurred in the osmotic pressure of any of the solutions, excepting the solutions of glucose, which showed a well marked rise; this rise agrees with the hypothesis already outlined. According to this hypothesis most of the other solutions should have shown a slight fall in osmotic pressure, no such fall was observed, but the readings of the freezing point were only accurate to the nearest 0.005°C. and therefore I do not consider this negative result of importance.

TABLE 4

The changes in osmotic pressure undergone by solutions, which were approximately isotonic when introduced, during their stay in the peritoneal cavity

SUBSTANCE	NUMBER OF EXPERIMENTS	Δ OF FIELD WHEN INTRODUCED	Δ OF FLUID WHEN REMOVED	
			I After one hour	II After three hours
Sodium chloride.....	15	0.560	0.574	0.560
Sodium chloride.....	3	0.560		
Sodium chloride plus epinine.....	3	0.560	0.574	
Glucose.....	6	0.557	0.596	0.588
Glucose.....	3	0.585		
Glucose plus epinine.....	5	0.550	0.596	
Sodium iodide.....	3	0.555	0.555	
Sodium sulphate.....	4	0.560	0.550	
Sodium acetate.....	2	0.540	0.565	
Sodium nitrate.....	2	0.520	0.517	

A study of the absorption of sodium chloride solutions confirms the view that the absorption of fluid was determined by the rate of diffusion for sodium chloride solutions were absorbed more rapidly during the first hour than in subsequent hours. For instance in small rabbits 14.8 per cent of fluid was absorbed in the first hour and only 29 per cent in three hours, again in large rabbits 23 per cent of the fluid was absorbed in the first hour, but when the fluid which had been removed at the end of the hour was injected into other rabbits only 16 per cent of it was absorbed in an hour. Therefore the nearer the sodium chloride

solution approaches in composition to the blood, and the nearer the rate of diffusion of its constituents approaches that of the blood constituents, the more slowly it is absorbed.

It is difficult to determine precisely the average rate of diffusion of the substances that produce the osmotic pressure of the blood, but since rabbits plasma has a Δ of -0.590°C . and it contains about 0.6 per cent NaCl which produces a Δ of -0.370°C ., and the remainder of the osmotic pressure (corresponding to a Δ of -0.220°C .) must be produced by more slowly diffusible substances (carbonates, phosphates, sugar, etc.), therefore the average rate of diffusion must be about 20 per cent less than that of NaCl, or even lower if some of the NaCl is in combination with the proteins of the plasma.

SUMMARY

The variations in the rate of absorption from the peritoneal cavity of fluid and solute from the different solutions investigated can be explained most simply as follows.

As soon as the fluid is introduced, free diffusion occurs between the fluid in the peritoneal cavity, and the blood, or the tissue fluids, and an equilibrium is reached when the peritoneal fluid contains 0.6 per cent NaCl, and a quantity of other substances, including proteins, which together produce a depression of the freezing point of about 0.2°C . This process of exchange is hastened by vaso-dilatation and slowed by vaso-constriction. The more rapidly the solute in the peritoneal fluid diffuses the more rapidly is this equilibrium attained, that is to say, the more rapidly is the solute absorbed.

At the same time absorption of the fluid may occur, and the rate of absorption is determined by the relation between the rate of diffusion of the substances producing the osmotic pressure of the blood, and the rate of diffusion of the substances in the peritoneal fluid. When, for instance, a solution of sodium chloride is introduced, the rate of diffusion of the chloride is greater than the average rate of diffusion of the constituents of the blood, and a fairly rapid absorption of fluid occurs, but by the end of an hour various slowly diffusible substances have entered

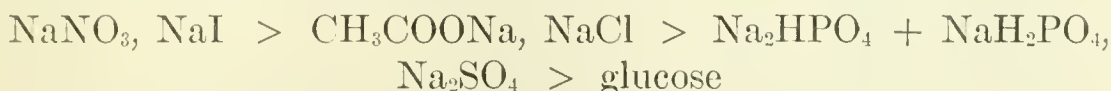
the peritoneal fluid from the blood stream, and absorption becomes slower.

On the other hand; if a substance such as glucose is introduced, which has a very low rate of diffusion, then, since the rate of diffusion of the substance in the peritoneal fluid is lower than the rate of diffusion of the constituents of the blood, salts enter the peritoneal fluid quicker than they leave it, the osmotic pressure of the fluid rises, and little or no absorption takes place; after an hour much of the glucose has left the peritoneal cavity, but absorption still remains very slow, and the osmotic pressure is still higher than that of the blood.

No attempt is made to explain the absorption of fluids which are in complete equilibrium with the blood, such as serum, but the absorption of such fluids is comparatively slow, and any absorption due to these unknown causes would be masked, in the experiments described, by the more rapid changes due to the differences in the rate of diffusion.

CONCLUSIONS

1. When isotonic solutions of different salts are injected intraperitoneally into rabbits, the rate of absorption of both water and solute in the different solutions is in the following order:



2. In the case of solutions of sodium chloride and glucose, the rate of absorption of both water and solute is increased by the presence of sodium fluoride.

3. The rate of absorption of water from a solution of sodium chloride is increased when the temperature of the fluid injected is raised to 45°C. The rate of absorption of both water and salt is diminished, when the temperature of the fluid is reduced to 25°C.

4. Epinine slightly increases the rate of absorption of water from solutions of sodium chloride and glucose.

5. When fluid, that has been in the peritoneal cavity of a rabbit for an hour, is taken out, and injected into a second ani-

mal, then the rate of absorption of both salt and water proceed more slowly in the second, than in the first animal.

6. The rate of absorption of solutions of both sodium chloride and glucose diminishes after the first hour.

7. When a solution of glucose is injected intraperitoneally, about 75 per cent of the glucose is absorbed in three hours, but none of the water is absorbed, and usually a larger volume of fluid is removed than was injected.

8. I attribute these variations in the rate of absorption to variations in the ratio between the rate of diffusion of the substances in the peritoneal fluid and the rate of diffusion of the substances in the tissue fluids, or the blood.

These experiments were performed in part at the Pharmacological Department, Guy's Hospital, London, and in part at the Pharmacological Department, University of Cape Town.

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Protocols

I. The absorption of sodium chloride

In all cases unless otherwise stated, 100 cc. of 0.95 per cent sodium chloride ($\Delta = -0.560^{\circ}\text{C}.$) were injected at $37^{\circ}\text{C}.$ into the peritoneal cavity of a rabbit, and the fluid was removed after one hour.

NUMBER OF EXPERIMENT	WEIGHT OF RABBIT	VOLUME OF FLUID REMOVED	COMPOSITION OF FLUID REMOVED		PERCENTAGE ABSORBED	
			I Per cent NaCl	II Δ	I Fluid	II NaCl

i. Effect of varying the weight of the rabbit

a. Small rabbits; weight 700 to 1000 grams

24	1000	88.0	0.84	0.559	12.0	22.0
34	920	88.0	0.80	0.550	12.0	26.0
41	1000	78.0	0.79	0.584	22.0	34.8
42	770	83.0	0.81	0.574	17.0	29.5
43	880	77.0	0.825	0.586	23.0	33.0
44	820	86.0	0.72	0.573	14.0	35.0
47	820	94.0	0.85	0.570	6.0	16.0
48	790	88.0	0.84	0.564	12.0	22.0
Average	877	85.2	0.81	0.572	14.8	27.5

b. Large rabbits; weight 1500 to 2000 grams

54	1950	85.0	0.83	0.580	15.0	26.0
85	1670	72.0	0.81	0.550	28.0	39.0
86	1700	77.0	0.81	0.570	23.0	34.0
87	1650	71.0	0.75	0.555	29.0	44.0
88	1800	69.0	0.73	0.570	31.0	47.0
91	1500	80.0	0.78	0.610	20.0	34.0
97	1500	85.0	0.84	0.600	15.0	25.0
Average	1680	77.0	0.793	0.576	23.0	35.6

ii. Effect of varying the temperature of the fluid injected

a. Fluid introduced at $25^{\circ}\text{C}.$

26	1260	90.0	0.81	0.558	10.0	23.0
38	1300	79.0	0.73	0.560	21.0	39.0
39	1120	94.0	0.78	0.560	6.0	23.0
40	1100	90.0	0.81	0.560	10.0	23.0
45	1190	89.0	0.87	0.574	11.0	18.5
46	1225	89.0	0.83	0.564	11.0	22.0
49	1200	83.0	0.77	0.556	17.0	32.5
50	1300	97.0	0.78	0.590	3.0	20.0
61	1315	95.0	0.78	0.580	5.0	22.0
92	1420	81.0	0.77	0.540	19.0	34.0
93	1350	91.0	0.84	0.545	9.0	20.0
Average	1250	89.8	0.797	0.562	11.2	25.0

NUMBER OF EXPERIMENT	WEIGHT OF RABBIT	VOLUME OF FLUID REMOVED	COMPOSITION OF FLUID REMOVED		PERCENTAGE ABSORBED	
			I Per cent NaCl	II Δ	I Fluid	II NaCl
b. Fluid introduced at 45°C.						
62	1850	60.0	0.80	0.558	40.0	49.5
63	1750	65.0	0.84	0.578	35.0	42.5
64	1500	81.0	0.82	0.578	19.0	30.0
94	1300	74.0	0.88	0.590	26.0	31.5
Average.....	1600	70.0	0.835	0.576	30.0	38.4
iii. The effect of varying the time that the fluid was left in the peritoneal cavity						
a. Fluid left in for 30 minutes						
1	1600	91.0	0.87	0.570	9.0	17.0
10	1261	91.0	0.77	0.540	9.0	26.0
17	1385	92.0	0.87	0.534	8.0	16.0
18	1425	95.0	0.90	0.550	5.0	10.5
19	1530	86.0	0.89	0.565	14.0	19.5
20	1122	93.0	0.91	0.575	7.0	11.5
21	1210	97.0	0.89	0.579	3.0	9.5
22	885	80.0	0.91	0.570	20.0	22.5
25	760	74.0	0.92	0.578	26.0	28.4
Average.....	1240	88.8	0.88	0.562	11.2	17.8
b. Fluid left in for 45 minutes						
11	2004	84.0	0.813	0.564	16.0	28.5
23	960	82.0	0.86	0.559	18.0	26.0
Average.....	1480	83.0	0.836	0.561	17.0	27.2
c. Fluid left in for 180 minutes						
35	960	68.0	0.74	0.560	32.0	47.5
36	882	72.0	0.735	0.550	28.0	44.0
37	822	74.0	0.73	0.565	26.0	43.0
Average.....	880	71.0	0.735	0.560	29.0	44.8
iv. The effect of the addition of sodium fluoride						
The fluid injected contained 0.85 per cent NaCl and 0.10 per cent NaF						
65	1960	66.0	0.63	0.590	34.0	51.0
66	2150	68.0	0.67	0.574	32.0	46.5
95	1510	90.0	0.575	0.585	10.0	39.0
Average.....	1870	74.7	0.625	0.583	25.3	45.5

NUMBER OF EXPERIMENT	WEIGHT OF RABBIT	VOLUME OF FLUID REMOVED	COMPOSITION OF FLUID REMOVED		PERCENTAGE ABSORBED	
			I Per cent NaCl	II Δ	I Fluid	II NaCl

v. The effect of the addition of epinine. Two doses of 0.25 cc. of 1 per cent epinine were injected intraperitoneally, the first at the commencement of the experiment, and the second after 30 minutes

67	1800	60.0	0.70	0.581	40.0	56.0
83	1650	78.0	0.93	0.566	22.0	23.5
84	1520	82.0	0.81	0.576	18.0	30.0
Average.....	1970	73.3	0.81	0.574	26.7	36.5

II. The absorption of fluid taken from the peritoneal cavity of a rabbit

100 cc. of 0.95 per cent sodium chloride solution was injected intraperitoneally into several rabbits, after an hour, the fluids were removed and mixed, and 109 cc. of the mixed fluid was injected into a rabbit.

NUMBER OF EXPERIMENT	WEIGHT OF RABBIT	COMPOSITION OF FLUID INTRODUCED		VOLUME OF FLUID REMOVED	COMPOSITION OF FLUID REMOVED		PERCENTAGE ABSORBED	
		I Per cent NaCl	II Δ		I Per cent NaCl	II Δ	I Fluid	II NaCl

a. Fluid left in peritoneum for 60 minutes

30	1320	0.86	0.554	85	0.75	0.566	15	25.5
51	1300	0.72	0.530	76	0.75	0.540	24	20.8
53	1620	0.72	0.520	85	0.60	0.510	15	20.8
96	1650	0.82	0.571	90	0.72	0.570	10	15.5
98	1420	0.77	0.585	83	0.78	0.540	17	25.5
Average	1510	0.76	0.552	84	0.72	0.544	16	21.9

b. Fluid left in for 180 minutes

31	1050	0.72	0.540	78	0.75	0.540	22	18.0
32	1350	0.72	0.540	98	0.68	0.538	2	7.6
33	1850	0.72	0.540	79	0.66	0.530	21	27.8
Average	1420	0.72	0.540	85	0.69	0.536	15	17.8

III. The absorption of glucose solutions from the peritoneal cavity

100 cc. of glucose solution, approximately isotonic, was injected intraperitoneally into rabbits.

NUMBER OF EXPERIMENT	WEIGHT OF RABBIT	COMPOSITION OF FLUID INJECTED		VOLUME OF FLUID REMOVED	COMPOSITION OF FLUID REMOVED			PERCENTAGE AB- SORBED	
		I Per cent glucose	II Δ		I Per cent glucose	II Per cent NaCl	III Δ	I Fluid	II Glucose
a. Solution left in peritoneal cavity for 60 minutes									
5	1440	5.7	0.593	109	3.13	0.327	0.605	-9	40.5
58	1900	5.2	0.540	104	2.85	0.415	0.610	-4	43.2
59	1750	5.2	0.540	104	3.00	0.485	0.610	-4	40.5
73	1520	5.3	0.576	98	2.70	0.376	0.576	2	50.0
80	2000	5.2	0.547	85	2.67	0.395	0.595	15	56.5
81	1370	5.2	0.547	106	3.17	0.326	0.583	-6	35.4
Average.	1590	5.3	0.557	101	2.92	0.387	0.596	-1	44.3
b. Solution left in peritoneal cavity for 180 minutes									
9	938	5.83	0.610	105	2.21	0.410	0.585	-5	60.5
15	1735	5.4	0.570	108	1.27	0.515	0.592	-8	74.5
16	1695	5.4	0.570	92	0.98	0.538	0.587	8	83.3
Average.	1456	5.54	0.583	102	1.48	0.488	0.588	-2	72.8
c. Solution left in for 60 minutes: 1 cc. of 0.1 per cent hemisine injected intra- peritoneally every 15 minutes									
72	1620	5.3	0.556	95	2.47	0.398	0.586	5	55.5
75	1370	5.3	0.556	89	3.9	0.240	0.596	11	34.6
77	1820	5.0	0.547	85	3.02	0.387	0.602	15	50.5
79	1800	5.0	0.547	85	3.17	0.330	0.587	15	48.5
82	1320	5.0	0.547	80	3.08	0.352	0.610	20	52.8
Average.	1586	5.1	0.550	87	3.13	0.341	0.596	13	48.4
d. A solution of glucose plus 0.1 per cent sodium fluoride injected									
74	1300	4.8	0.586	96	1.57	0.370	0.576	4	69.0
76	1550	4.8	0.586	87	1.96	0.405	0.582	13	64.2
78	1770	5.0	0.627	87	1.47	0.564	0.597	13	74.5
Average.	1524	4.9	0.596	90	1.66	0.445	0.585	10	69.2

IV. *The absorption of isotonic solutions of various salts from the peritoneal cavity of the rabbit*

100 cc. of solution was injected at a temperature of 37°C. and left in for an hour.

NUMBER OF EXPERIMENT	WEIGHT OF RABBIT	VOLUME OF FLUID RE- MOVED	COMPOSITION OF FLUID REMOVED			PERCENTAGE ABSORBED	
			I Per cent salt	II Percent NaCl	III Δ	I Fluid	II Salt
a. 1.85 per cent sodium sulphate anhydrous injected; $\Delta = -0.560^{\circ}\text{C}$.							
12	1756	96.0	0.85	0.286	0.552	4	56.0
56	1300	72.0	1.20	0.248	0.530	28.0	53.5
57	1500	90.0	1.10	0.295	0.552	12	46.5
89	1670	88.0	0.93	0.367	0.566	10	55.0
Average.....	1555	86.5	1.02	0.299	0.550	13.5	52.7
b. 1.3 per cent sodium nitrate injected; $\Delta = -0.520^{\circ}\text{C}$.							
103	1700	74.0	0.425	0.415	0.520	26	75.8
104	1800	59.0	0.461	0.340	0.515	41.0	79.0
Average.....	1750	66.5	0.443	0.378	0.517	33.5	77.4
c. 2.05 per cent sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) injected; $\Delta = -0.540^{\circ}\text{C}$.							
105	1900	73.0	0.954	0.502	0.560	27.0	66.0
106	1800	79.0	0.816	0.375	0.570	21.0	68.7
Average.....	1850	76.0	0.885	0.438	0.565	24.0	67.3
d. 2.40 per cent sodium iodide injected; $\Delta = -0.555^{\circ}\text{C}$.							
99	1540	77.0	0.824	0.434	0.565	23.0	73.5
101	1500	66.0	0.614	0.526	0.555	34.0	83.0
102	1400	83.0	0.974	0.345	0.545	17.0	66.2
Average.....	1480	75.0	0.804	0.435	0.555	25.0	74.2
e. Mixture of acid and alkaline sodium phosphate injected; total concentration of phosphate equals 0.17 mol.; $\Delta = -0.620^{\circ}\text{C}$.							
107 (pH = 7.0)	1500	75.0	0.610 (P_2O_5)	0.405		25.0	62.6
108 (pH = 7.0)	1500	90.0	0.615	0.295		10.0	49.6
109 (pH = 6.0)	1450	96.0	0.618	0.465		4.0	51.3
110 (pH = 9.0)	1550	85.0	0.435	0.415		15.0	69.7
Average.....	1500	86.5	0.569	0.396		13.5	58.3
f. 4.82 per cent magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) injected; $\Delta = -0.440^{\circ}\text{C}$.							
3	1620	90.0	Mg = 0.346 SO ₄ = 1.470	0.258	0.555	10.0	Mg = 34 SO ₄ = 30.8

ON THE ABSORPTION OF LOCAL ANESTHETICS THROUGH THE GENITO-URINARY ORGANS¹

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INTRODUCTORY

Local anesthetics are employed extensively in genito-urinary practice. They are administered for the performance of minor operations, such as circumcisions, urethrotomies, etc., and are instilled into the urethra and bladder to relieve pain and facilitate the introduction of instruments, such as sounds, endoscopes, cystoscopes, dilators, etc. In gynecological practice, also, local anesthetics are applied to the urethra and bladder for the same purposes; and occasionally cocain suppositories are introduced even into the vagina for the relief of itching or other irritating or painful conditions.

In connection with a comparative study of local anesthetics in relation to the genito-urinary organs, which was undertaken by the author at the suggestion of the Council for Pharmacy and Chemistry of the American Medical Association, the author inquired into the question of absorption of these substances through the genito-urinary organs. In the present paper it is proposed to report in brief the results of this investigation.

Inasmuch as the possibility of absorption of local anesthetics from the genito-urinary tract is of interest chiefly from the toxicological point of view, that is, in reference to the question of whether these substances are absorbed in sufficient quantities

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to produce toxic symptoms, the experiments in this investigation were confined for the most part to the most important, as well as one of the most poisonous drugs belonging to this class, namely cocain. The other local anesthetics which were investigated in this respect were alypin and apothestin. The comparative toxicity of local anesthetics has been studied by various authors and in particular has been gone into recently very carefully by Eggleston and Hatcher (1) and from the work of the latter it is evident that among the more toxic local anesthetics which are employed in genito-urinary work are the three substances just mentioned. For this reason the present experiments were made with only these drugs.

ABSORPTION FROM THE BLADDER AND URETHRA

Method. In a communication on the absorption of apomorphin and morphin from the urethra and bladder (2), the author called attention to the fact that those drugs were admirably suited for the study of absorption through unusual channels or portals of entry, on the one hand, and that they could conveniently be employed for the study of the comparative degree of absorption of drugs from the urethra and bladder, on the other hand. In the present research the same method of study was employed.

The study of absorption from bladder and urethra was confined to male animals. Dogs were found to be ideal subjects for this purpose, because, in the first place, the histological structure of the urethra and bladder in the dog is practically the same as that in man, and secondly, because the vesical and urethral sphincters in this animal are so powerfully developed that it is possible to confine the action of a drug to the bladder or urethra at the pleasure of the experimenter.

If a fine catheter, with a side opening and a wire guide, is passed through the urethra of a male dog into its bladder, and is left in position, the internal sphincter contracts so firmly around it that any fluid injected through the catheter into the bladder is practically confined to that cavity alone, and does not permeate into the urethra. Indeed, in some dogs it is almost

impossible to pass a catheter into the bladder at all on account of the excessive irritability and spasmodic contraction of the sphincter muscles.

If, on the other hand, it is desired to limit the action of a drug to the urethral mucosa only and not the bladder, all that is necessary is to insert the catheter as far as the external sphincter, in which case a fluid injected through the catheter under moderate

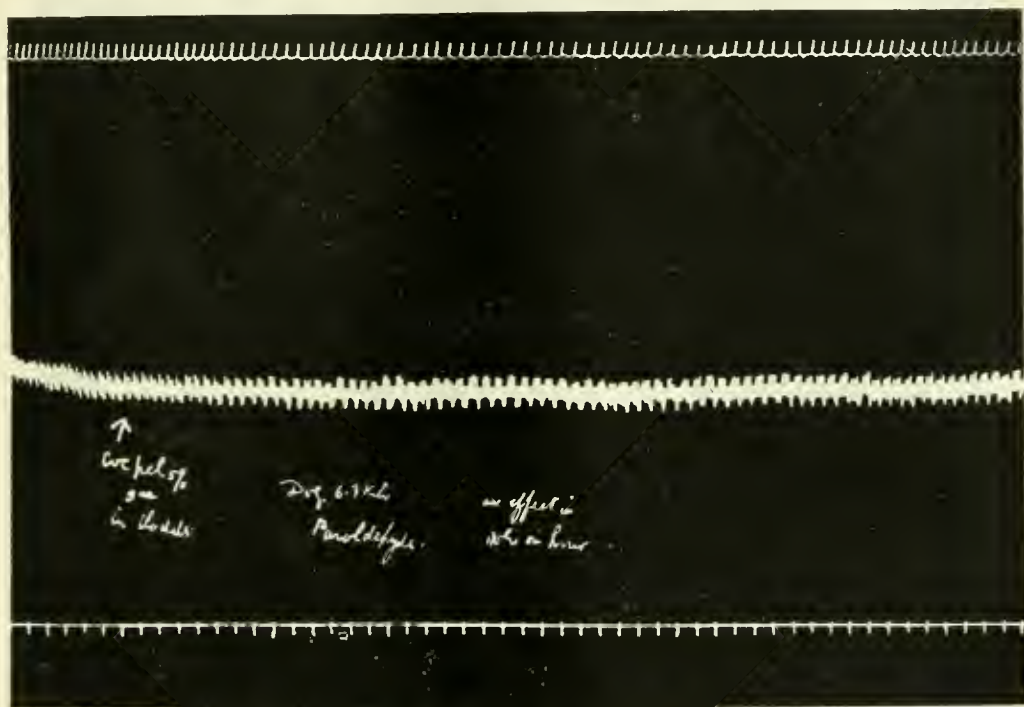


FIG. 1. DOG, 61.8 KILO. PARALDEHYDE ANESTHESIA

Five cubic centimeters of cocain hydrochloride (5 per cent solution) in bladder. Shows no effect on blood pressure or respiration after a period of over one hour.

pressure will merely irrigate the urethra and flow out again externally without penetrating into the bladder. The conditions just described hold good for the great majority of dogs. In exceptional cases, of course, the sphincters may not be so well developed or may not contract so tightly, thus preventing a sharp differentiation in applying the drug to the bladder or urethra.

Action of cocain. The relative absorption of cocain from the bladder and urethra is strikingly illustrated by figures 1 and 2.

In figure 1, 5 cc. of cocain hydrochlorid (5 per cent solution) was introduced into the bladder of a dog and retained in the same by the method just described, for a period of over one hour. It will be noted that there was absolutely no effect produced on the respiratory and blood-pressure curves. In figure 2 the urethra of the same dog was irrigated slowly with a 2 per

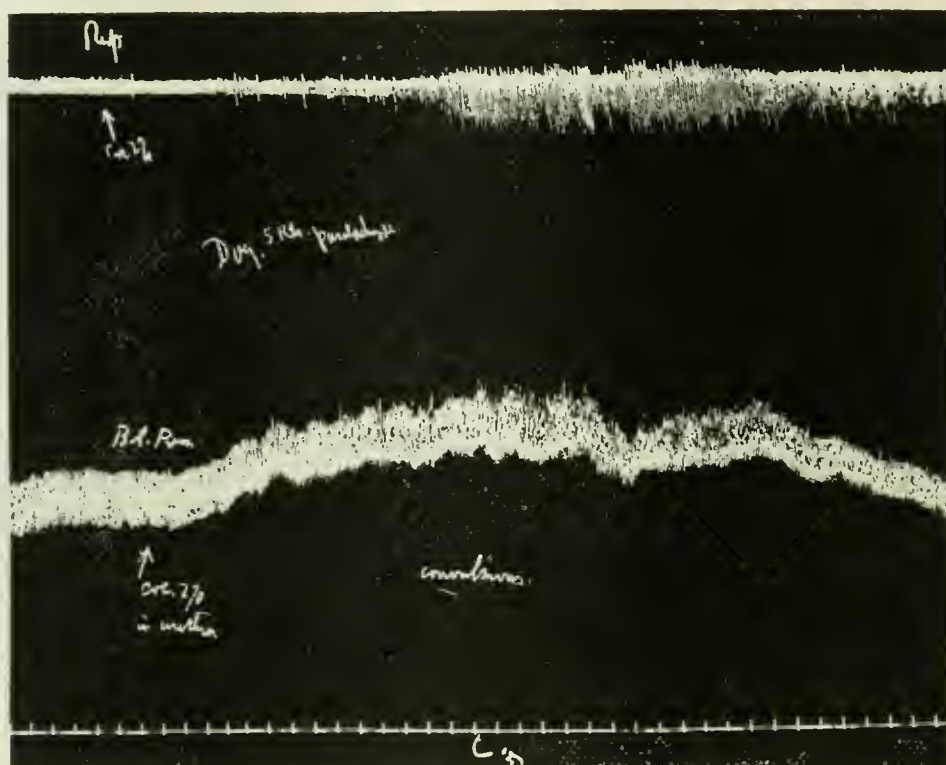


FIG. 2. DOG, 5 KILO. PARALDEHYDE ANESTHESIA

Showing the effect of irrigating the urethra with a 2 per cent solution of cocain hydrochloride. Note the rapid and marked effect on blood pressure and respiration.

cent solution of cocain hydrochloride and very soon after beginning the irrigation definite signs of absorption were manifested. The blood pressure was changed and the respiration was markedly stimulated, as it generally is at first by small doses of cocain. Following these symptoms the dog developed convulsions, showing a deeper poisoning, and this was followed gradually by a fall in blood pressure and shallow respirations. It is thus evident

that absorption of cocain from the urethra takes place very readily, whereas the same drug is but very poorly absorbed through the walls of the bladder.

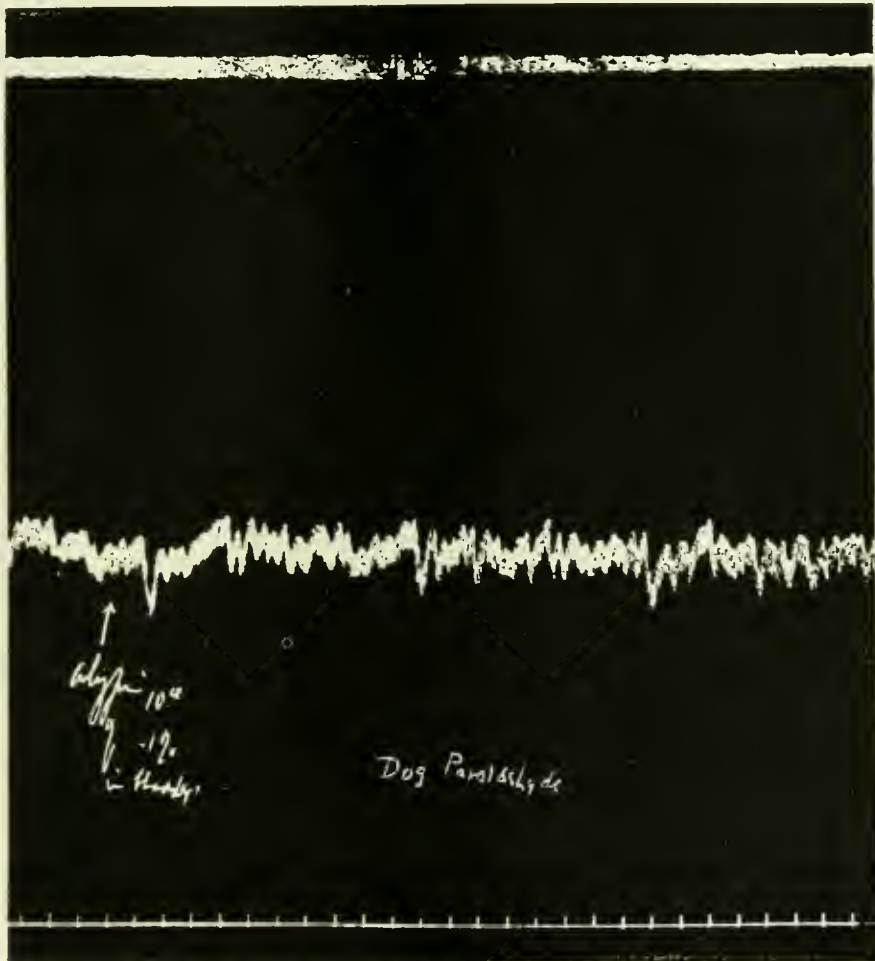


FIG. 3. EFFECT OF ALYPIN ON THE ABSORPTION FROM THE BLADDER

Ten cubic centimeters of a 0.1 per cent solution produces no effect after 1 hour

Action of alypin and apothetin. Experiments with alypin and apothetin (the hydrochlorid of diethyl-amino-propyl cinnamate) gave results analogous to those obtained with cocain. These drugs were also much more readily absorbed from the urethra than from the bladder (see figures 3 and 4).

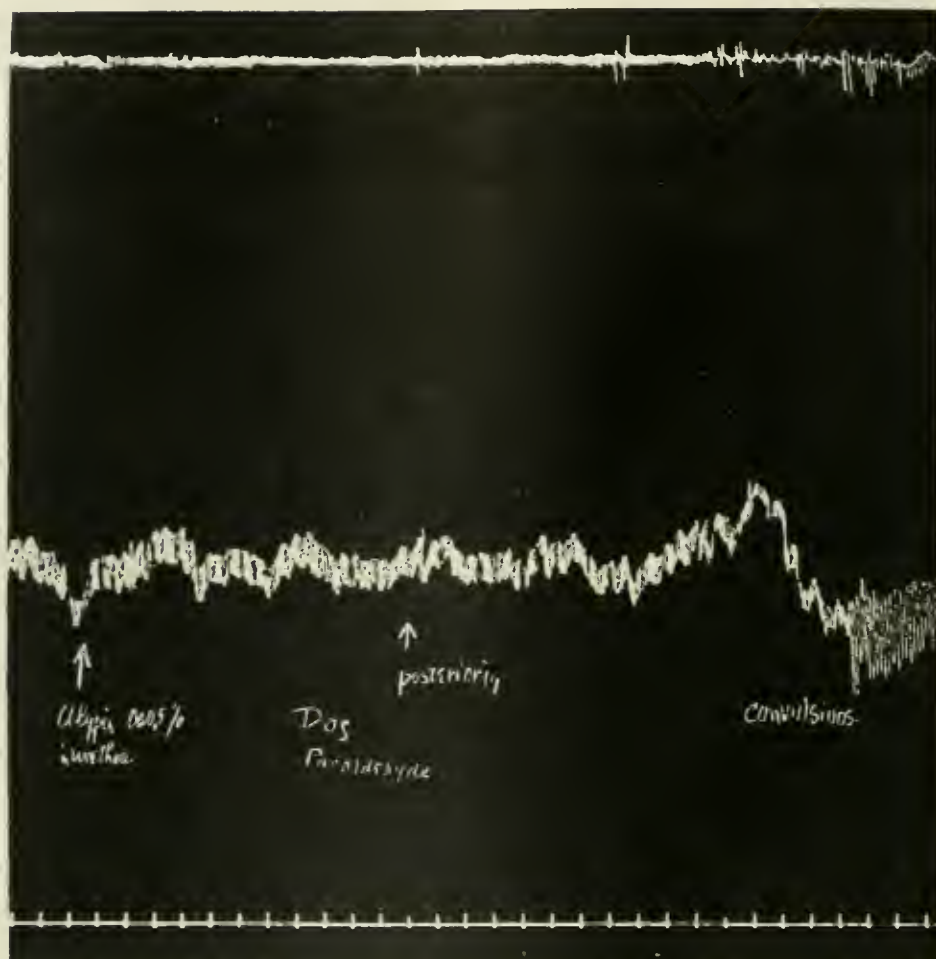


FIG. 4. ABSORPTION OF ALYPIN FROM URETHRA

Effect of irrigation with a 0.05 per cent of the drug. Upper curve, respiration; lower curve, blood pressure.

ABSORPTION FROM THE URETER AND PELVIS OF THE KIDNEY

Method. Absorption from the ureter and kidney pelvis was studied in dogs. In order to exclude the possibility of error through absorption through the peritoneum and other channels, the following procedure was followed:

An incision was made just below the floating ribs and the kidney exposed. A glass cannula was then inserted into the ureter just below the kidney pelvis pointing distalward. A fine ureteral catheter was passed through the ureter from above downward and its insertion in the bladder located. An incision was then made in the abdominal wall just above the bladder

and another cannula, with a small rubber tube attached, was inserted into the lower end of the ureter pointing upward. Particular pains were taken to ligate the cannulae tightly so as to prevent leakage of the perfusing fluid. The whole length of the ureter was thus perfused with a saline solution by injecting the fluid under low pressure into the upper cannula and allowing the solution to escape from the lower cannula and rubber tubing without coming in contact with the peritoneum or entering the

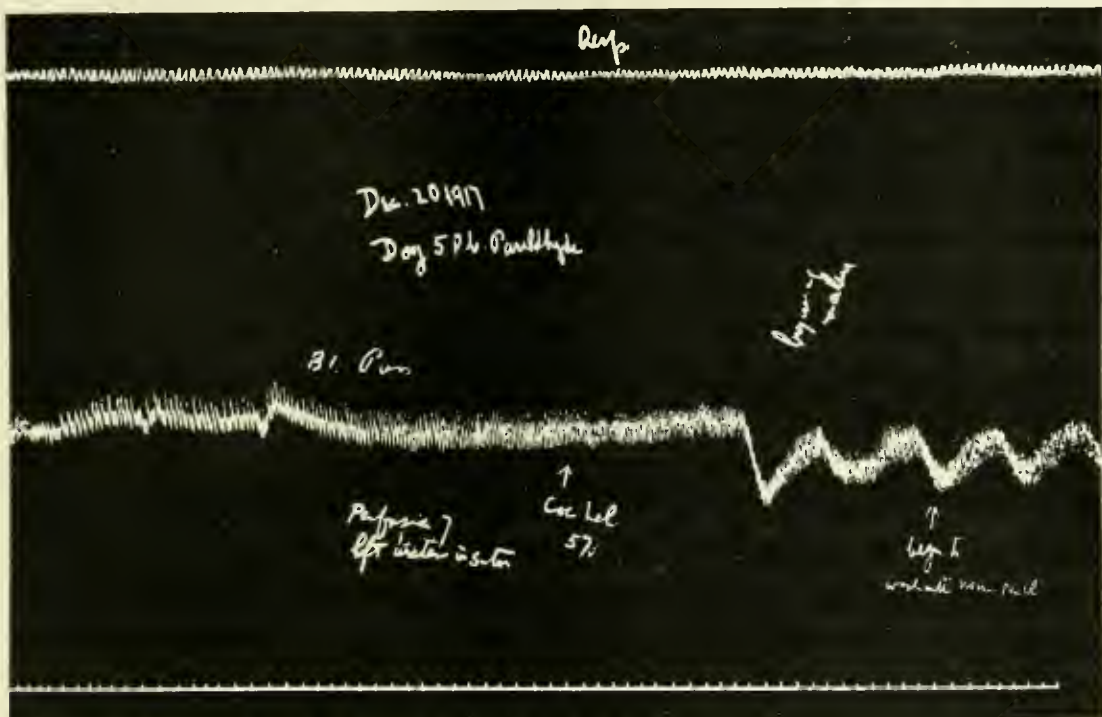


FIG. 5. ABSORPTION OF COCAIN HYDROCHLORIDE FROM URETER OF THE DOG
Showing the effect of perfusion with a 1 per cent solution

bladder. In this way normal physiological saline solutions and saline solution containing the drug to be studied could be perfused through the ureter alone and the absorption of drugs or poisons studied at ease.

In order to study absorption of drugs from the kidney pelvis the procedure in the first steps was the same as above. An incision being made below the floating ribs, the kidney was exposed and its pelvis freed from surrounding structures. A good sized glass cannula was then inserted into the pelvis and

ligated pointing upward or toward the kidney. A fine ureteral catheter was then inserted loosely inside of the glass cannula and the perfusing fluid injected through the fine catheter, and allowed to flow back through the larger glass cannula surrounding it. A drug in solution could thus be brought in contact with the pelvis of the kidney under low pressure and the fluid allowed to run back around the catheter through the glass cannula without coming in contact with the peritoneum or surrounding tissues.

Effect of cocain. Experiments with cocain performed by the above method showed clearly that that drug is absorbed readily both through the ureteral walls and through the pelvis of the kidney. In figure 5 the left ureter of a dog was perfused slowly with a 1 per cent solution of cocain hydrochloride in physiological saline. The dog was anesthetized with paraldehyde and the respiratory and blood pressure curves were recorded. It will be seen that perfusion with cocain produced a fall in blood pressure and convulsions, thus indicating that the drug was absorbed.

ABSORPTION THROUGH THE PRAEPUTIUM

A careful search of the literature by the author has failed to reveal any experiments bearing on the subject of absorption through the praeputium penis. An investigation was therefore begun by the author concerning the absorption of different classes of drugs through that organ. In this place only the experiments with local anesthetics are recorded, while a fuller paper bearing on the subject in general will be published in the *Journal of Urology*.

For the purpose of experimentation the dog is the most suitable animal, as the praeputium of that animal is highly developed. In the experiments performed the drugs were introduced into the preputial sac and the physiological effects pointing to its absorption into the general circulation were looked for. In case of the local anesthetics, the curves of respiration and blood pressure and convulsive movements were especially noted.

It was found that a number of local anesthetics which were examined along with other drugs were readily absorbed through



FIG. 6. DOG, 8 KILO. PARALDEHYDE ANESTHESIA

Showing effect of introducing two tablets of apothesis in the praeputial sac.
Upper curve, respiration; lower curve, blood pressure.

the praeputium. Figure 6 shows this phenomenon in a striking manner. After cocain, fluctuations in the respiration and blood pressure, as well as convulsive movements of the muscles, were noted. After apothecin, very rapid poisoning of the animal occurred, following the introduction of two tablets of the drug into the praeputial sac.

ABSORPTION THROUGH THE VAGINA

An experimental study of the subject of absorption of drugs and poisons through the vagina, as well as a review of the clinical toxicological literature of the same, was published in this journal

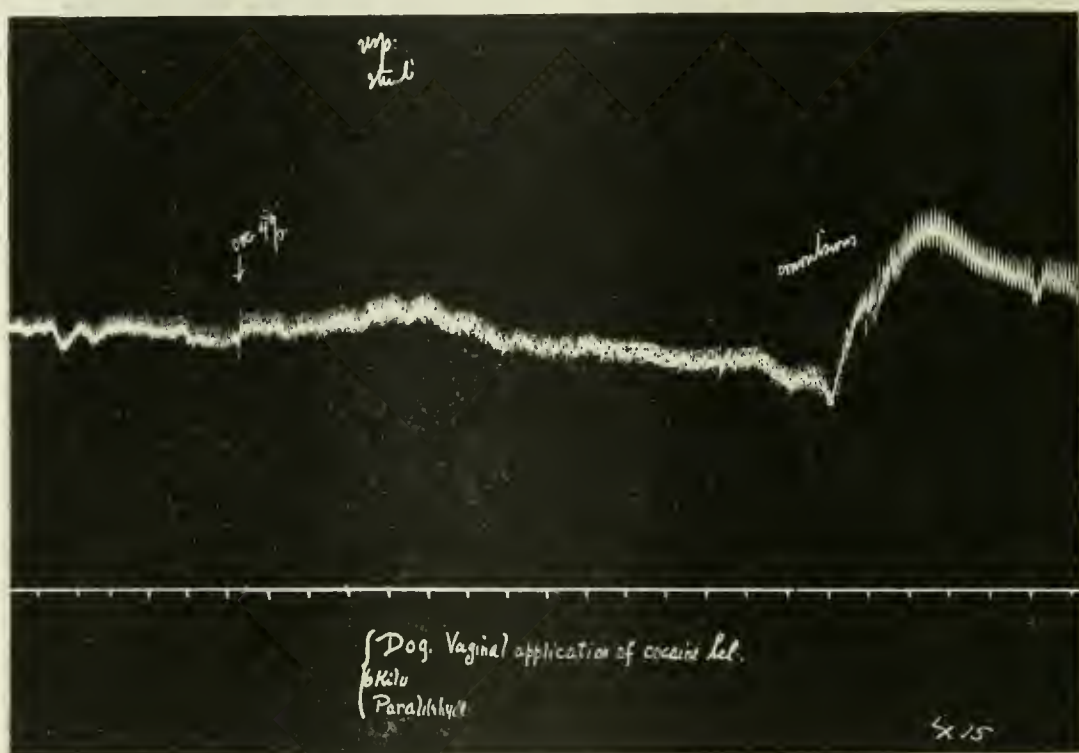


FIG. 7. DOG, 6 KILO. PARALDEHYDE ANESTHESIA

Five cubic centimeters of a 4 per cent solution of cocaine hydrochloride was introduced into vagina. Note effect on blood pressure and convulsions. The respiratory curve is not shown in this figure.

by the author (3) sometime ago. In the present communication, reference need be made only to the absorption of local anesthetics by that route.

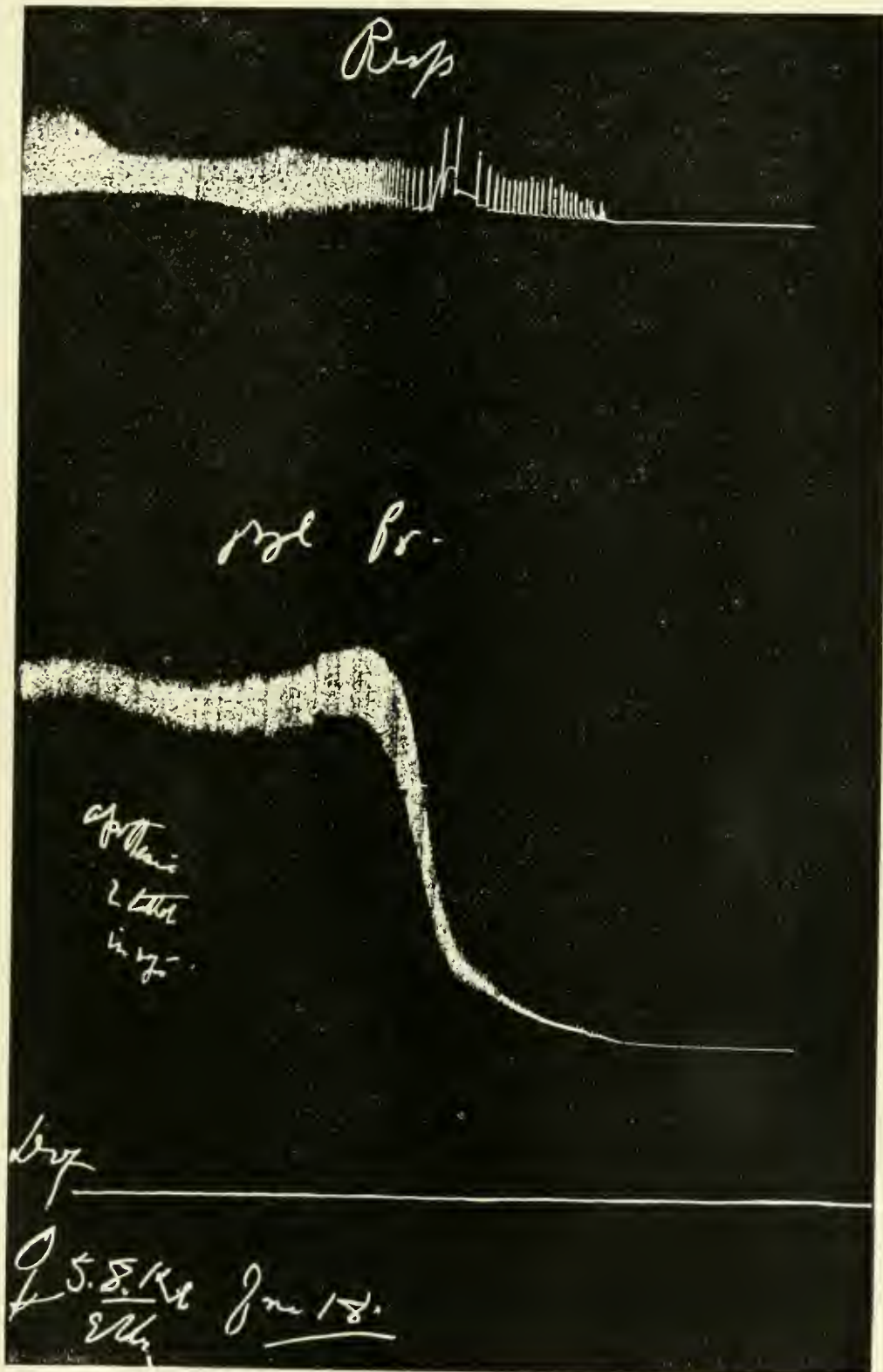


FIG. 8. DOG, 5.8 KILO. ETHER ANESTHESIA

Showing effect which followed the introduction of two tablets of apothesis into the vagina.



FIG. 9. DOG, 10 KILO. PARALDEHYDE ANESTHESIA

At the points indicated by arrows, 4 cc. of a 1 per cent solution of apothesisin was injected intravenously.

That local anesthetics are readily absorbed through the vaginal wall is readily illustrated, in figures 7 and 8. In figure 7, a 4 per cent solution of cocain hydrochloride, 5 cc., was introduced into the vagina of a dog. It will be noted that after the lapse of a few minutes, constitutional symptoms of poisoning developed. The respirations were first stimulated and then depressed (not shown in the figure); the animal developed convulsions; and the blood pressure curve shows first a drop and later a rise, due to the convulsive contractions of the muscles.

Figure 8 illustrates the effect of introducing two tablets of apothecin into the vagina of a dog. It is interesting to compare this figure with figure 9, in which 4 cc. of a 1 per cent solution of apothecin were injected intravenously in another dog. It will be seen that the poisonous effect of the drug is almost as marked in the first case as in the second.

DISCUSSION

As a result of the various experiments recorded above, it is evident that local anesthetics are very easily absorbed through various organs of the genito-urinary tract, with the exception of the bladder. Of great scientific as well as practical interest is also the difference between the absorptive powers of the urethra as compared with the bladder. Whereas, drugs are very easily taken up through the urethral walls, they are very slowly absorbed, indeed, from the bladder. These experimental data agree very well with the experiences of both urologists and gynecologists, so far as the author has been able to ascertain. The absorption of cocain through the ureter and pelvis of the kidney is chiefly of scientific interest; and yet, in view of the modern use of drugs, such as iodides, thorium, etc., in these structures, the subject may also be of some practical importance. Absorption through the prepuce is important from the surgical point of view, as local anesthetics are very widely employed in this connection, and their careless or excessive use may lead, and has led, to toxic symptoms in patients. The absorption of local anesthetics from the vagina is also interesting, even though

these drugs are not so widely introduced into that canal as other poisons are, such, for instance, as the antiseptics mercuric chloride, phenol, lysol, etc.

SUMMARY

1. The local anesthetics, cocain, alypin and apothetin, were studied in regard to their penetration through and absorption from various génito-urinary organs.

2. It was found that these drugs are more or less readily absorbed through the urethra, ureters, pelvis of the kidney, praeputium and vagina.

3. It was found that while the local anesthetics are readily and rapidly absorbed from the urethra, they are very poorly absorbed from the urinary bladder.

REFERENCES

- (1) EGGLESTON AND HATCHER: Jour. Pharmacol. & Exp. Therap., 1919, xiii, 433.
- (2) MACHT: Jour. Urol., 1918, ii, 43.
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QUANTITATIVE STUDIES IN CHEMOTHERAPY

IV. THE RELATIVE THERAPEUTIC VALUE OF ARSPHENAMINE AND NEOARSPHENAMINE OF DIFFERENT MANUFACTURE

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I

Ehrlich and Hata (1) pointed out that the ratio of the therapeutic activity of arspenamine and neoarsphenamine on a basis of the absolute weight of the drugs was 0.6:0.9 as judged by experiments on hen spirillosis and mice infected with the spirochetes of relapsing fever. Since arspenamine contains on an average 30 per cent and neoarsphenamine 19 per cent arsenic, the ratio of activity of the arsenic in the two compounds would be 1:1.

Schamberg, Kolmer and Raiziss (2) have recently found a slightly greater ratio of 0.6:1.02, working with *Tr. equiperdum* in rats and using as a criterion, sterilization for a period of three weeks. This, with other evidence, is given below:

Ehrlich and Hata (1)	{ Hen spirillosis.....	0.6:0.9
	{ Sp. Obermayer, mice.....	0.6:0.9
Schamberg, Kolmer and Raiziss (2). <i>Tr. equiperdum</i> , rats....		0.6:1.05
Castelli (3)	{ Sp. Obermayer, mice.....	0.6:1.07
	{ Hen spirillosis.....	0.6:1.02
	{ Rabbit syphilis.....	0.6:0.9
Voegtlin and Smith,* <i>Tr. equiperdum</i>		0.6:1.02

* This communication.

¹ The authors are indebted to Miss M. A. Connell and Miss Lula Frère for assistance in this investigation.

There is little choice, and no need for argument, over the ratio. It is sufficiently near a mean to indicate that the arsenic in the two compounds has essentially the same activity, although clinical observers have repeatedly claimed that neoarsphenamine is less effective in the treatment of human syphilis than arsphenamine.

In a previous communication (4) a single preparation of arsphenamine and one of neoarsphenamine showed a trypanocidal ratio of 2:3 on the arsenic content, and a therapeutic ratio, or $\frac{\text{M. L. D.}}{\text{M. E. D.}}$ of 37:33. That this low trypanocidal dose for ars-

phenamine represents an exceptional case is brought out by a further study of several more preparations. These preparations are representative samples of leading manufacturers (one of both compounds being a German preparation imported several years ago). The minimum effective dose was determined by the method previously described (4) and the toxicity by the official method recommended by the United States Public Health Service (5).

For the determination of the M. E. D. the doses were graded upon a smaller scale than was used in our previous work. Rats having between 100,000 and 300,000 trypanosomes (*Tr. equiperdum*) per cubic millimeter of blood were used and were examined twenty-four hours after the injection and practically every day from then on through a period of sixty days. A minimum effective dose will just drive all or nearly all of the parasites from the blood stream. Sterilization is never complete at the minimum effective dose however, and the few remaining parasites multiply rapidly, the animal usually dying of trypanosomiasis in three to ten days. Our experience with a more detailed study of this phenomenon convinced us that the sharpness of this threshold is to be attributed to the fact that a minimum concentration of drug is necessary to effect the destruction of the majority of the parasites present in the blood stream, and that a relapse can be avoided only by going considerably above this dose.² Further

² The relation between M. E. D. and curative dose will be discussed in a subsequent paper.

experience has substantiated our conjecture that this method of determining the trypanocidal activity of a drug is superior to a study of relapses following this early "primary-reaction" period, and that results so obtained are more reliable. The method used by Schamberg, Kolmer and Raiziss is based upon sterilization of the animal, and yields results which vary by 300 per cent on the same preparation. With our method, we have had no difficulty in getting results consistently within 50 per cent. Consequently the efficacy of the various arsphenamine and neoarsphenamine preparations under investigation has been determined by the minimum effective dose procedure.

The results of the "primary-reaction" and the subsequent behavior of the infection have been briefly condensed in the following tables. The dose is indicated in cubic centimeters of 1:100 arsenic-equivalent solution per kilo body weight and was given intravenously. The results of an examination made twenty-four hours after the injection of the drug are recorded in the first column under each preparation; ++ indicates 1000 or more trypanosomes per cubic millimeter of blood; + indicates less than 1000. In the second column is given the day of death; here + simply indicates that trypanosomes were present in the blood at, or just previous to, the time of death, and - indicates that death was due to some other cause than trypanosomiasis. When no day of death is given, the animal survived twenty-eight days, the maximum period during which relapse can occur with the strain of trypanosomes used.

In table 3 the net results of tables 1 and 2 are tabulated with the minimum lethal doses. The arsphenamine preparations studied showed practically the same minimum effective dose though the minimum lethal dose varied nearly 100 per cent. An average of these figures gives a minimum effective dose of 3.1, minimum lethal dose 53.2, $\frac{\text{M. L. D.}}{\text{M. E. D.}} = 17.2$. The average figures

for neoarsphenamine are: M. E. D. = 3.4, M. L. D. 96.5, $\frac{\text{M. L. D.}}{\text{M. E. D.}} = 28.4$. The neoarsphenamine preparations showed a greater variation in the minimum effective dose and less in the minimum

lethal dose than did the arsphenamines, but in 4 out of 5 cases the ratio $\frac{\text{M. L. D.}}{\text{M. E. D.}}$ is greater than the highest ratio for any sample of arsphenamine. In agreement with Castelli and with Schamberg, et al., we find that the minimum effective dose of both

TABLE 1

DOSE: 1:100 As EQUIVALENT SOLUTION PER KILO	ARSPHENAMINE NO. 1		ARSPHENAMINE NO. 2		ARSPHENAMINE NO. 3		ARSPHENAMINE NO. 4		ARSPHENAMINE NO. 5	
	24 hours	Day of death	24 hours	Day of death	24 hours	Day of death	24 hours	Day of death	24 hours	Day of death
cc.										
1.0	+	4+	++	3+	++	4+	+	4+	++	2+
	++	4+	++	3+					+	8+
1.5	+	4+	+	4+	+	4+	+	5+	+	4+
	+	7	+	4-					+	4+
2.0	+	8-	+	4+	+		+		+	3+
	+	6-	+	7+						
2.5	+	6-	+	6?	+	9	+	4+	+	3+
	+	13+	+	6+					-	5+
3.0	-	6-	-	7-	+	9+	-	6-	-	4-
	-	6-	-	8-					+	4-
4.0	-	11-	-	4-	-	11+	-	11-	-	7-
	-	13-	-	23-					-	4-
4.5	-	9+		23+	-	11-	-	6-	-	3-
	-	9+								
5.0									-	7-

compounds is approximately the same, i.e., 3.4 cc. neoarsphenamine, and 3.1 cc. arsphenamine; by weight, the activity ratio of the two compounds would be 0.6:1.02.

In answer to the clinical claims that neoarsphenamine is less effective than arsphenamine we must call attention to the differences in the chemical and physical properties of the two compounds and in the nature of the diseases under consideration

(trypanosomiasis and syphilis). Swift (6) has made a detailed study of the rate of disappearance of arsenic from the site of injection following the intramuscular administration of arsphenamine and neoarsphenamine to rabbits. His results are tabulated

TABLE 2

DOSE: 1: 100 AS EQUIVALENT SOLUTION PER KILO	NEOARSPHENA- MINE NO. 1		NEOARSPHENA- MINE NO. 2		NEOARSPHENA- MINE NO. 3		NEOARSPHENA- MINE NO. 4		NEOARSPHENA- MINE NO. 5	
	24 hours	Day of death	24 hours	Day of death	24 hours	Day of death	24 hours	Day of death	24 hours	Day of death
cc. 1.5							++	2+	++	2+
2.0	++	2+	++	4+	++	3+	+	4+	+	4+
	++	2+	++	4+	++	3+				
2.5	++	2+	++	4+	++	3+	—	—	—	3—
	++	4+	++	4+	+	12+				
3.0	++	2+	+	9+	—	10?	—	11+	++	3
	++	4+	++	5+	—	10?				
3.5	++	4+	+	11+	+	5—	—	11+	—	5—
	++	2+	+	12+	—	5+				
4.0	++	5+	—	5—	—	9+	—	9+	—	4—
	+	5+	—	7—	—	12+				
4.5	—	10+					—	—	—	5—
	—	17+								
8.0	—	4—	—	4—	—	—			—	5—
	—	4—	—	—	—	—				
12.0	—	—	—	5—	—	—				
	—	—	—	1—	—	—				

briefly in table 4. The figures give the average percentage absorption as determined on several animals.

At the end of the first week more than twice as much neoarsphenamine had been absorbed as of arsphenamine. Lockemann (7) studying the rate of excretion of various arsenicals found that neoarsphenamine was excreted more rapidly than arsphenamine,

though with both compounds excretion was extremely slow. Theoretical considerations would lead one to expect that arspphenamine would be retained in the body for a longer time than neoarsphenamine, for the former is precipitated at the hydroxyl

TABLE 3

	PREPARATION NUMBER	PERCENT- AGE ARSENIC	1:100 As EQUIVA- LENT SOLUTION PER KILO BODY WEIGHT		M. L. D. M. E. D.	M. L. D. PER KILO BODY WEIGHT
			M. E. D.	M. L. D.		
Arsphenamine.			cc.	cc.		mgm.
	1	31.53	3.0	50.5	16.8	120
	2	29.99	3.0	40.0	13.3	100
	3	31.35	4.0	41.8	10.5	100
	4	30.55	3.0	61.0	20.4	150
	5	32.00	3.0	51.2	17.0	120
	6	31.48	3.0	75.0	25.0	180
	Average..	31.15	3.1	53.2	17.2	128
Neoarsphenamine...	1	19.49	(4.5)			
	2	18.79	(4.0)			
	3	19.96	3.0	96.0	32.0	360
	4	18.41	2.5	73.8	29.5	300
	5	19.73	2.5	116.0	46.4	440
	6	17.05	4.5	100.0	22.2	440
	Average..	18.90	(3.4)	96.5	32.5	385

TABLE 4

	WEEK							
	1	2	3	4	5	6	8	10
Salvarsan:								
Alkaline solution.	36.0	51.0	72.5	63.5		69.5		86.0
Neutral suspension...	28.5	54.0	71.5	71.5		78.0		89.5
Acid suspension.....	30.0	54.0	57.5	48.0		79.5	87.0	
Neosalvarsan.....	80.5	88.5		90.5	92.5	95.0		

ion concentration of the blood (8) and consequently must become temporarily fixed in a nonreactive state in the tissues.³ Little

³ In this connection Myers' (9) figures are interesting. He found that thirty minutes after the intravenous administration of 0.4 gram arspphenamine, only 25 per cent of the injected arsenic is found in the blood.

by little this deposition is probably oxidized to the parasitocidal oxide, and is distributed throughout the body by the blood stream. Neoarsphenamine, on the other hand, is not precipitated at the hydroxyl ion concentration of the blood, and as a result its oxidation and elimination probably proceeds at a greater rate.

So far as rat trypanosomiasis, hen spirillosis and relapsing fever in mice are concerned, the relative rates of excretion of the drugs should be of less importance than in human syphilis. In the former, the majority of the parasites are in the blood stream and there is little evidence of deep seated lesions not readily reached through a single intravenous dose. In the latter, there are fewer parasites in the blood, and more in the deep seated foci in tissues which have undergone chronic change, and these will certainly not be so easily reached. Getting the drug to these foci is largely a matter of time, and consequently if the reactive oxide formed from neoarsphenamine disappears more rapidly through excretion (and possibly oxidation) than the corresponding oxide derived from arsphenamine, the former compound will have less opportunity of reaching and sterilizing these recesses, and in the same doses (on an arsenic basis) will prove less effective.

The fact that the average therapeutic ratio $\frac{\text{M. L. D.}}{\text{M. E. D.}}$ is greater for neoarsphenamine than for arsphenamine (28.4 to 17.2 respectively) cannot be construed in our estimation as an experimental recommendation for giving larger doses of neoarsphenamine than is the custom at the present time. And since the two compounds appear to possess about equal protozoicidal activity, arsenic for arsenic, intravenously there is no evidence here for attributing any advantages to neoarsphenamine except that the dose necessary to kill the patient, if comparable to rats, is slightly farther removed from the therapeutic dose. The greater instability of neoarsphenamine towards oxidation (10) and the consequent danger of increased toxicity, and its possible decreased effectiveness in human syphilis, add further emphasis to this point. So far as the experimental evidence is concerned, we feel

that a choice between the two drugs must be based upon clinical results, and upon the practical advantages and disadvantages in the use of the two preparations.

II. SUPPLEMENTARY DATA ON ARSENICALS

In 1899 Gautier (11) introduced sodium cacodylate into therapeutics as a substitute for inorganic arsenic, principally on account of the low toxicity of this substance and the fact that it could be administered subcutaneously without causing any local irritation. This drug has been used very extensively ever since in the treatment of a great variety of diseases. It was soon found, however, that the administration of cacodylic acid is followed by the reduction of the drug to cacodyloxide which can be readily detected by the disagreeable odor which it imparts to the patient's breath. Because of this disagreeable feature and for other reasons, Gautier later recommended methyl arsenic acid (arrhenal). Mouneyrat (12) studied the toxicity and biological behavior of this substance and reported a number of case histories which he thought demonstrated the value of the drug in the treatment of malaria, syphilis, asthma, diabetes, and chorea. Soon after the discovery of arsphenamine, Murphy (13) claimed to have had some success in the treatment of syphilis by means of intramuscular injections of cacodylate. Wright, Kennell and Hussey (14) quite recently have reported that ethyl arsenic acid is also of therapeutic value in the treatment of this disease.

In view of these statements, it was of interest to subject these various aliphatic arsenicals to an experimental test as to their trypanocidal action, particularly as Nichols and Castelli (15) seem to have been the only investigators who had previously done experimental work along this line. Castelli's observations led him to the conclusion that neither cacodylic acid nor ethyl arsenic acid exhibited any action whatsoever on experimental trypanosomiasis and spirochete infections.

The work here to be reported was also undertaken with a view of furnishing additional proof for the conclusion reached by us from the study of the aromatic arsenicals, viz., that only the arsen-

icals of the trivalent oxide type (RAs:O) are directly protozoicidal. Incidentally a few additional aromatic arsenicals were included in this study.

The technique in testing the trypanocidal action of these compounds was the same as previously used. The results obtained will be found in table 5. Those compounds indicated by italics are reported for the first time. They were prepared and analyzed for us by Mr. J. W. Thompson of this laboratory. The diphenylarsenious oxide and triphenylarsine are insoluble in alkalis and it was necessary to use a finely divided suspension made by the addition of water to an alcoholic solution of these drugs. In this case it was impossible to give larger doses than 20 cc. per kilo (1:100 As equivalent solution) because of the rapidity with which the suspension crystallized in the metallic hypodermic needle.

Some of the new compounds presented in table 5 warrant some discussion. Ethyl arsenious oxide unlike all the other oxides of arsenic (with the exception of cacodyl oxide) is an amber colored liquid with a sweet and slightly offensive odor. It is insoluble in water, but soluble in a sufficient excess of alkali to form the theoretical disodium salt, $\text{C}_2\text{H}_5\text{As}(\text{ONa})_2$. In the pure state it is a very powerful skin irritant, producing a painful erythema in one to two hours. This is followed by marked and diffuse swelling which ends at the end of two days in a deep blister. Its vesicant action is analogous to that of phenyldichlorarsine and the related mustard gases. In working with it over a long period of time, Mr. Thompson developed a dermatitis similar to the chronic arsenic dermatitis so much in evidence with the chemists who worked with the chlorarsine war gases. Ethyl arsenious oxide is the only one of the arsenious oxides which shows this remarkable irritating property. Both ethyl arsenious oxide and methyl arsenious oxide give rise to an acute pulmonary edema but the latter compound, strange to say, has the lesser toxicity.

As to the trypanocidal properties of these compounds, it will be seen that the results described in table 5 are in agreement with the premises that compounds of the oxide type act directly upon both the parasites and the host, while the pentavalent compounds

TABLE 5

TYPE		COMPOUNDS ANALYZED	OBSERVED ARSENIC <i>per cent</i>	CALCULATED ARSENIC <i>per cent</i>	MINIMUM LETHAL DOSE	MINIMUM EFFECTIVE DOSE	M. L. D. M. E. D.
R. AsO R. AsO	Arsenious acid <i>Methyl arsenious oxide</i>	As ₂ O ₆ Na ₄ CH ₃ .AsO	45.6 70.7	46.5 70.8	7.0 3.75	7.0 3.75	1 ea 1 ea
R. AsO R. AsO	<i>Ethyl arsenious oxide</i> Phenyl arsenious oxide	C ₂ H ₅ AsO(H ₂ O?) C ₆ H ₅ .AsO	59.0 45.38	62.5 44.6	5.0 3.75	2.25 0.75	2 5
R. AsO	<i>p</i> -Aminophenyl ar- senious oxide	p-NH ₂ .C ₆ H ₄ .AsO.2H ₂ O	34.1	34.1	1.5	0.75	2
4 5 R. AsO	m-Amino- <i>p</i> -oxy- phenyl arsenious oxide	m-NH ₂ - <i>p</i> -OH.C ₆ H ₃ .AsO	27.37	31.82	10.0	0.75	13
(R ₂ As) ₂ O	<i>Diphenyl arsenious oxide</i>	((C ₆ H ₅) ₂ As) ₂ O		31.5	7.5	5.0	1 ea
R ₃ As R. AsO(OH) ₂ R. AsO(OH) ₂ R. AsO(OH) ₂	<i>Triphenyl arsine</i> <i>Methyl arsenic acid</i> <i>Ethyl arsenic acid</i> N-phenyl glycine amide p-arsenic acid	(C ₆ H ₅) ₃ .As CH ₃ .AsO(ONa) ₂ .6H ₂ O C ₂ H ₅ .AsO(OH) ₂ p-NH ₂ .CO.CH ₂ .NH.C ₆ H ₄ .AsO(ONa) ₂	25.2 35.34 24.38	25.6 48.70 25.0	>20.0 375.0 200.0 750.0	>20.0 375.0 200.0 75.0	1 ea 1 ea 1 ea 10
R. AsO(OH) ₂	Phenylglycine- <i>p</i> -ar- senic acid	p-COOH.CH ₂ .NH.C ₆ H ₄ .AsO(OH) ₂	27.17	27.27	750.0	375.0	2
R. AsO(OH) ₂	<i>p</i> -Aminophenyl ar- senic acid	p-NH ₂ .C ₆ H ₄ .AsO(ONa) ₂ .2H ₂ O	27.67	27.2	150.0	37.5	4

R. AsO(OH) ₂	m-Amino-p-oxy phenyl acid	m-NH ₂ p OH. C ₆ H ₃ . AsO(OH) ₂	28.82	32.40	225.0	37.5	6
R. AsO(OH) ₂	Arsacetine	CH ₃ . CO. NH. C ₆ H ₄ . AsO(OH). ONa. 4H ₂ O	21.16	21.24	750.0	37.5	20
R. AsO(OH) ₂	Arsenic acid	AsO ₄ Na ₃ . 4H ₂ O	31.3	30.80	50.0	37.5	1 ca
R ₂ AsO(OH)	<i>Cacodylic acid</i>	(CH ₃) ₂ . AsO(ONa). 3H ₂ O	33.3	35.0	1000.0	1000.0	1 ca
R. As:As. R	Neoarsphenamine*	m-NH ₂ -p-OH. C ₆ H ₃ . As:As. C ₆ H ₃ . p-OH-m-NH. CH ₂ OSONa	18.90		96.5	3.4	28
R. As:As. R	Arsphenamine*	m-NH-2-p-OH. C ₆ H ₃ . As:As. C ₆ H ₃ -p-OH-m-NH ₂	31.15		53.2	3.1	17
R. As:As. R	Arsenophenylglycine	p-COOH. CH ₂ . NH. C ₆ H ₄ As:As. C ₆ H ₄ . NH. CH ₂ . COOH	35.20		75.0	4.5	16

*Average figures.

must be converted by reduction to the corresponding oxides before they exert their principal toxic action. Experiments which are not reported here in detail have shown that the trypanosome count of infected rats begins to diminish immediately after the injection of a minimum effective dose of the oxides, thus proving that these substances act in exactly the same way as the aromatic oxides.⁴ That the AsO group is the essential group in this toxic action is brought out by a comparison of the minimum effective dose of these compounds with their molecular size. There is an increased activity with increased molecular size, a relation which would be expected from other studies dealing with the relation between chemical constitution and pharmacological action. The nature of the substituting radicals exerts no modifying action upon the trypanocidal activity, a finding which is contrary to the view held by Ehrlich that the side chains represent specific chemoceptors and are involved in the action of the drug.

The results obtained with sodium cacodylate, which, as has been noted, has been recommended in times past as a substitute for arsphenamine, show that it does not exert any protozoicidal action even in lethal doses, and must be, therefore, considered as a quite worthless preparation for this purpose. The minimum effective dose of ethyl arsenic acid, like the other pentavalent arsenicals, is very high, and the therapeutic ratio is lower than with the majority of these compounds. It is inferior, so far as rat trypanosomiasis is concerned, to several of the other pentavalent compounds, and it is therefore inconceivable how it could be of superior or even equal clinical value.

SUMMARY

1. Six different brands of arsphenamine, including a German preparation, have shown approximately the same trypanocidal activity.

2. Slightly greater variations in activity were observed with six different samples of neoarsphenamine, the maximum difference being 80 per cent.

⁴ Methyl and ethyl arsenic acid showed a marked latent period during which the parasite count was not altered.

3. There appears to exist no relation between toxicity and trypanocidal action of arsphenamine and neoarsphenamine.

4. It is suggested that the alleged greater effectiveness of arsphenamine over neoarsphenamine in the treatment of human syphilis may be attributed to the fact that arsphenamine is precipitated at the hydroxyl ion concentration of the blood, in consequence of which the rate of its oxidation and elimination from the body is considerably diminished.

5. The study of the trypanocidal action of some aliphatic arsenicals has confirmed the fundamental principle formulated from the previous study of the aromatic arsenicals, viz., that the trivalent oxides are the only forms of arsenic which exert a direct toxic action upon protoplasm.

6. Cacodylic acid does not possess any trypanocidal action even in lethal doses. Methyl and ethyl arsenic acid show a parasitocidal action only when used in doses approaching the lethal dose.

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STUDIES OF CHRONIC INTOXICATIONS ON ALBINO RATS

III. ACETIC AND FORMIC ACIDS

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INTRODUCTION

The immediate occasion for investigating these acids was the question of the safety of the use of formic acid for the preservation of foods. The supposed safety of acetic acid for similar purposes made this an admirable measure of comparison; so that parallel experiments were carried out with both acids. However, the investigation was planned with a view to possible wider applications.

The effects of continued use of acetic acid have not been experimentally studied. It seemed of interest to learn whether the continued ingestion of considerable quantities of acid influenced digestion and metabolism, for instance by direct effects on the digestive tract, which is so largely controlled by the acid and alkaline reactions.

Acetic acid is so readily oxidized that it would probably not affect the acid-base equilibrium of the tissues. The oxidation of formic acid is less complete (Fleig), and might therefore conceivably alter the equilibrium, and this would probably be reflected in growth.

Formic acid has a further interest as an oxidation product of methyl alcohol (Pohl). Chronic or subacute poisoning with formic acid, if it exists, might therefore throw some light on chronic or subacute methyl alcohol poisoning.

Finally, some fifteen or eighteen years ago, formic acid and formates were loudly proclaimed as a sort of elixir of youth, destined to redeem the world, and to confer on men the indefatigability of insects. This action, if it existed, should be reflected in the nutrition and growth.

The interesting history of this fantastic propaganda as well as its scientific refutation, are given in the comprehensive paper of Fleig, 1907. He showed the remarkable tonic effects to be merely imaginary. The descriptions of his experiences are worth reading; for they apply equally to more modish tonics and rejuvenants; especially interesting is the description of the asthenic patient (page 215) who recovered his vigor on sodium chlorid, that he mistook for the more advertised formate.

Fleig's paper also contains the scientific data as to formates, of which his own are the most important. He reviews the literature so thoroughly that it is not necessary to enter further into that subject; except to abstract very briefly the data that apply to the present investigation. There seems to have been very little scientific work with formates since Fleig.

METHODS

The experiments were conducted as described in the first and second papers of this series; the acids being added to the drinking water, so that they were consumed continuously.

Dosage. This is determined by the concentration of the drug and by the thirst of the animals. The mean doses are shown in table 1. The weekly variations appear unimportant. They could be calculated from the curve of fluid consumption.

For the preservation of food, 0.1 to 0.15 per cent of free formic acid is said to suffice (Lebbin). This falls within the limit of the concentration (0.01 to 0.5 per cent) of the present experiments.

Lebbin administered to men for four weeks, daily doses of 0.5 gram of formic acid per kilogram of body weight, without any effects. This would correspond to about 8 mgm. per kilogram, which is our smallest dosage. Our largest dosage is 45 times as great. Lebbin also tried doses larger than 0.5 gram. They produced only local actions, analogous to acetic acid. In rabbits, continued administration of either formic or acetic acid damaged the kidneys.

Of sodium formate, Fleig found the acute toxic dose for dogs to be 3 grams per kilogram by vein, 4 grams per kilogram by mouth; this would correspond to 250 grams for man; i.e., the toxicity of the formate is of the same order as that of sodium chlorid. Men consumed daily doses of 10 grams of sodium formate, equivalent to 150 mgm. per kilogram, for some time without any harm. Eppinger observed hematuria in one out of three patients taking 3 to 4 grams per day; but this looks like an accidental coincidence.

TABLE 1

Dosage of acetic and formic acids

CONCENTRATION OF ACID	EXPERIMENT NUMBER	NUMBER OF ANIMALS IN EX- PERIMENT	DURATION	MEAN DOSAGE, MILLIGRAMS OF ACID PER KILOGRAM OF RAT PER DAY	
Acetic acid					
<i>per cent</i>			<i>weeks</i>		
0.01	56	6	11	8.2	(5.7-11.3)
0.01	107	3	9	10.9	(9.6-12.9)
0.1	63	6	15	81.0	(63.0-95.0)
0.25 (after 10 weeks of 0.01)	5695	4	15	210.0	(79.0-390.0)
0.5 (after 16 weeks of 0.1)	6305	3	9	360.0	(310.0-710.0)
Formic acid					
0.01	57	6	11	8.2	(5.6-12.1)
0.01	108	3	14	10.25	(8.4-14.3)
0.1	64	6	15	90.0	(80.0-131.0)
0.25 (after 12 weeks of 0.01)	5796	4	15	160.0	(120.0-210.0)
0.5 (after 17 weeks of 0.1)	6406	3	9	360.0	(290.0-670.0)

CONSUMPTION OF FLUID

Table 2 gives the mean daily consumption of fluid in each experiment; as well as the mean for the first, middle and last third of each experiment, so as to indicate the influence of the continuance of the experiment.

Table 2 shows that there are no distinctive differences in the fluid consumption as between acetic or formic acids, or the different concentrations, or the different periods. It may there-

fore be concluded that neither acetic nor formic acid affect the consumption of fluid when added to the drink in concentrations up to at least 0.5 per cent.

TABLE 2
Mean daily consumption of fluid

ACIDS	EXPER- IMENT NUM- BER	DURA- TION	FLUID CONSUMPTION (CUBIC CENTIMETERS PER KILOGRAM OF RAT)			
			Entire period	First third	Middle third	Last third
		<i>weeks</i>				
Acetic acid, 0.01 per cent	56	11	82.0 (57-113)	104	80	80
Acetic acid, 0.01 per cent	107	9	109.0 (96-129)	106	112	107
Acetic acid, 0.1 per cent	63	15	81.0 (63-95)	83.5	69	80
Acetic acid, 0.25 per cent	5695	15	90.0 (74-155)	106	88	90
Acetic acid, 0.5 per cent	6305	9	71.0 (63-142)	71	67	86
Formic acid, 0.01 per cent	57	11	82.0 (56-121)	101	75	62
Formic acid, 0.01 per cent	108	14	102.5 (84-125)	122	89	99
Formic acid, 0.1 per cent	64	15	90.0 (80-109)	86	89	92
Formic acid, 0.25 per cent	5796	15	64.0 (49- 85)	64	62	66
Formic acid, 0.5 per cent	6406	9	72.0 (58-135)	73	58	82
Formic acid, 0.75 per cent	138	2	127.0 (116-138)			

DIURETIC ACTION

The fact that formic acid does not increase the consumption of fluid implies that it does not act as a diuretic. According to Fleig, formates had considerable reputation as diuretics; and he quoted from Huchard several cases of marked diuresis following the administration of formates to cardiac and renal patients. If these were not accidental coincidences, they must have been due to the alkaline element of the formates. Fleig himself attributes the supposed diuresis to renal vasodilation that he observed on intravenous injection of sodium formate in animals; but this renal effect was evidently due to hydremic plethora from the injection of the solution.

EFFECTS ON GROWTH

The data are shown in table 3 and in figures 1, 2 and 3. It may be recalled that in non-medicated groups of rats, the variation from the standard growth do not exceed -1 to $+1.8$ per cent per week. The last column of table 3 shows that both

TABLE 3
Effects of acetic and formic acid on growth

DRUG AND CONCENTRATION	DOSAGE, CUBIC CENTIMETERS PER KILOGRAM OF RAT PER DAY	EXPERIMENT NUMBER	DURATION	OBSERVED WEIGHT	NORMAL WEIGHT	DIFFERENCE IN GRAMS	DIFFERENCE IN PER CENT OF NORMAL WEIGHT	DIFFERENCE PER CENT PER WEEK
			<i>weeks</i>					
Acetic acid, 0.01 per cent . . .	8.2	56	11	175	250	-75	-30.0	-2.7
Acetic acid, 0.01 per cent . . .	10.9	107	9	232	230	+2	+0.43	+0.04
Acetic acid, 0.1 per cent . . .	81.0	63	15	304	295	+9	+3.2	+0.21
Acetic acid, 0.25 per cent (after 10 weeks of 0.01 per cent)	210.0	5695	5	209	212	-3	-1.4	-0.28
			15	215	258	-43	-16.0	-1.06
Acetic acid, 0.5 per cent (after 16 weeks of 0.1 per cent)	360.0	6305	1	264	312	-48	-15.0	-15.0
			5	270	315	-45	-14.0	-2.8
			9	240	319	-79	-24.0	-2.6
Formic acid, 0.01 per cent . . .	8.2	57	10	199	190	+9	+4.6	-0.46
			11	162	192	-30	-15.0	-1.3
Formic acid, 0.01 per cent . . .	10.25	108	14	160	177	-17	-9.0	-0.63
Formic acid, 0.1 per cent . . .	90.0	64	15	275	282	-7	-2.0	-0.13
Formic acid, 0.25 per cent (after 12 weeks of 0.01 per cent)	160.0	5796	2	165	168	-3	-1.8	-0.9
			4	222	175	+47	+26.0	+6.4
			10	235	191	+44	+23.0	+2.3
			15	216	197	+19	+9.0	+0.6
Formic acid, 0.5 per cent (after 17 weeks of 0.1 per cent)	360.0	6406	9	180	295	-115	-56.0	-6.7

acetic and formic acids, in concentrations of 0.1 and 0.25 per cent are generally within these limits, and therefore had no effect on growth. With each acid, one experiment with 0.01 per cent also comes within the limits; and one experiment exceeds the

limits. In view of the negative results with 25 times this concentration, this experiment was evidently a coincidence.

By doubling the concentration, that is, with 0.5 per cent acids, and a dosage of 0.36 cc. per kilogram, both acids produced immediate and progressive check of growth, averaging 2.6 per cent per week for acetic acid, and 6.7 for formic acid or total

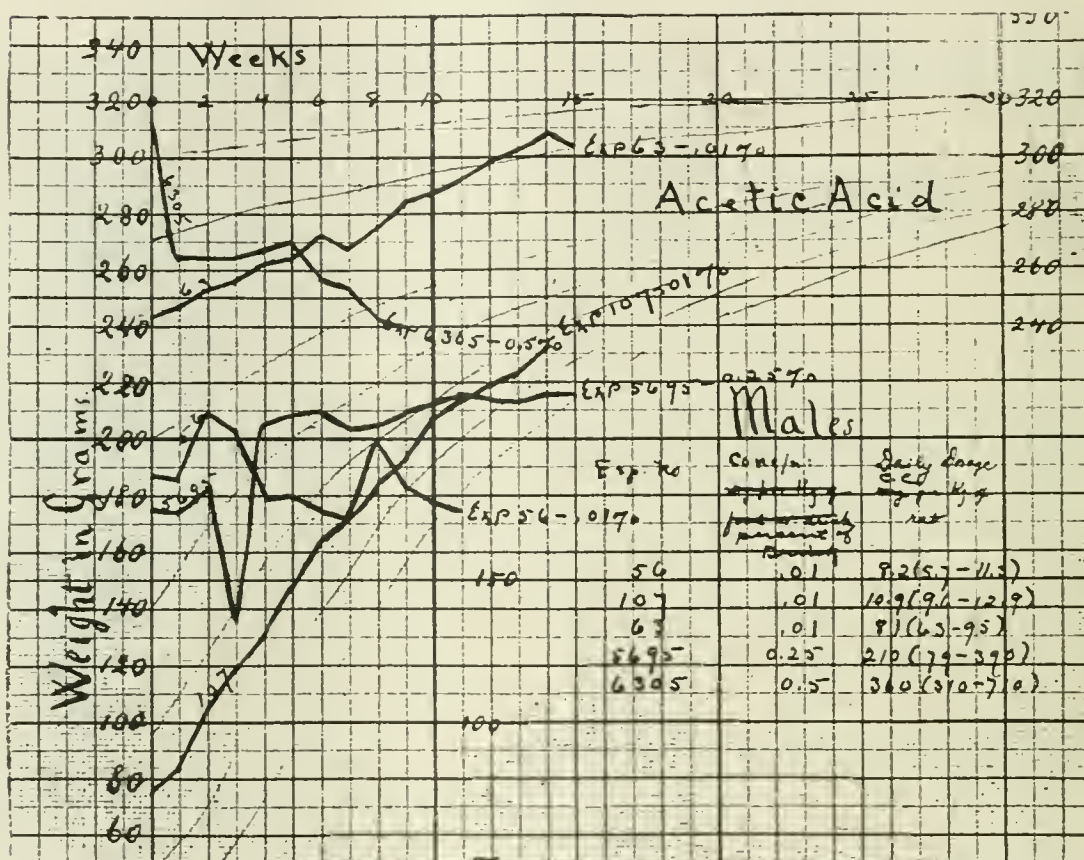


FIG. 1. EFFECT OF ACETIC ACID ON GROWTH

The heavy lines represent the growth of the alcohol rats; the light line represents the standard normal growth curves.

deficits, in the weight, of 24 per cent for acetic acid and 58 per cent for formic acid. The difference between the two acids is evidently accidental; for similar differences, but in the opposite direction, occur with the lower concentrations.

It may therefore be concluded that formic and acetic acid are quantitatively alike in their influence on growth. Neither has any effect in fifteen weeks when the concentration in the drink

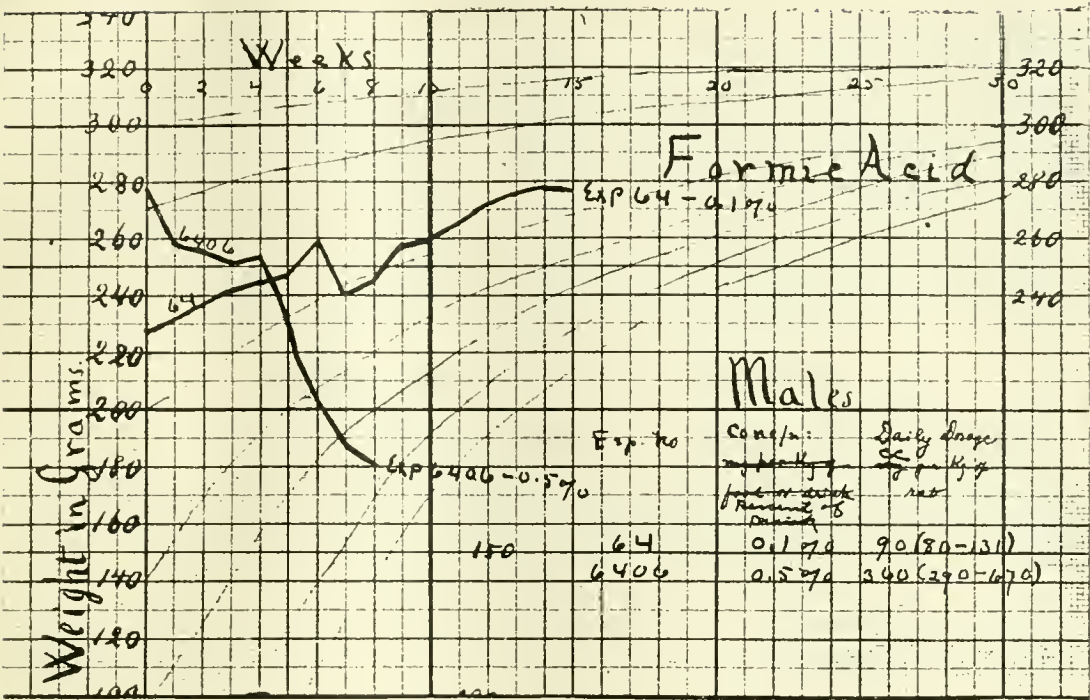


FIG. 2. FORMIC ACID ON GROWTH OF MALE RATS

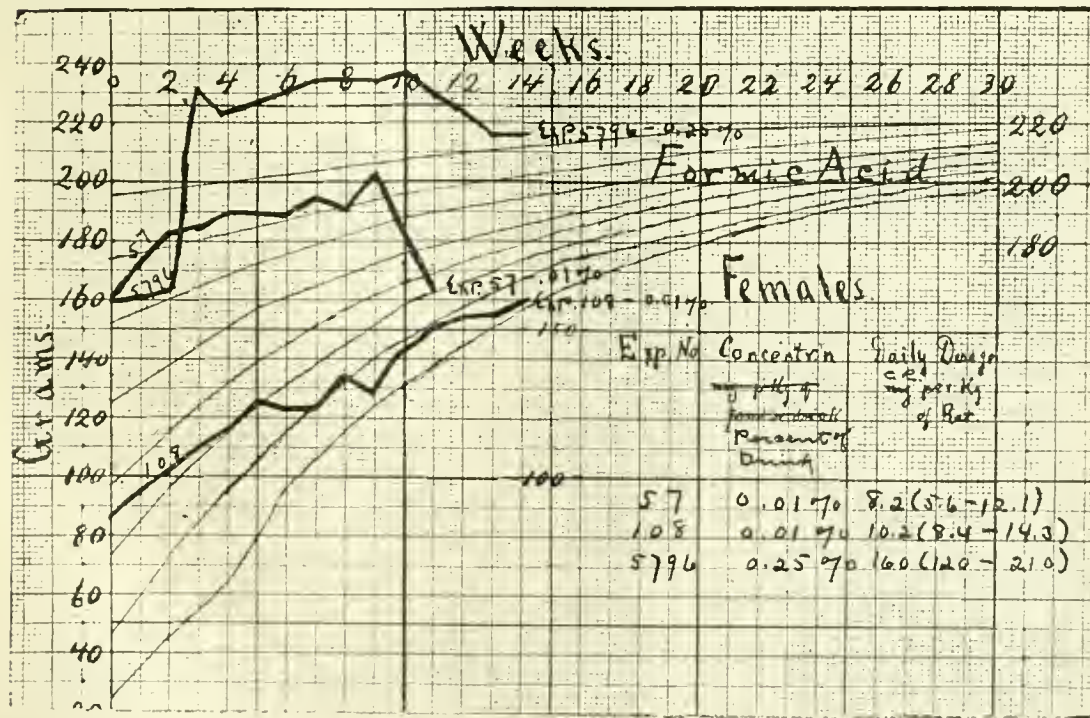


FIG. 3. FORMIC ACID ON GROWTH OF FEMALE RATS

does not exceed 0.25 per cent, with the daily dosage 0.2 cc. per kilogram of rat.

With a concentration of 0.5 per cent and daily dosage of 0.36 cc. per kilogram, growth is definitely checked from the start of the administration.

This does not depend on any change of the total fluid consumption, since this is not materially altered.

EFFECTS ON FOOD CONSUMPTION

These are shown in table 4 and figures 4 and 5.

In interpreting the data of table 4, it must be remembered that the extreme variation of unpoisoned rats from the standard

TABLE 4
Food consumption

ACIDS	EXPER- IMENT NUM- BER	DURATION OF EX- PERIMENT	GROWTH, MEAN DIFFERENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMP- TION, MEAN DIFFERENCE FROM NORMAL STANDARD GRAMS		MEAN DIFFERENCE (PER CENT) IN FOOD FOR SERIES
				Grams per rat per day	Per cent	
		<i>weeks</i>				
Acetic acid, 0.01 per cent	56	11	-2.7	-1.6	-15	} -5.5
Acetic acid, 0.01 per cent	107	9	+0.04	+0.45	+4	
Acetic acid, 0.1 per cent	63	15	+0.21	+0.2	+1	
Acetic acid, 0.25 per cent (after 10 weeks of 0.01 per cent)	5695	15	-1.06	-0.8	-7	+7.0
Acetic acid 0.5 per cent (after 16 weeks of 0.1 per cent)	6305	9	-2.6	-3.8	-27	-27.0
Formic acid, 0.01 per cent	57	11	-1.3	-0.4	-4	} -1.0
Formic acid, 0.01 per cent	108	14	-0.63	+0.2	+2	
Formic acid, 0.1 per cent	64	15	-0.13	+1.0	+8	
Formic acid, 0.25 per cent (after 12 weeks of 0.01 per cent)	5796	15	+0.6	+0.6	+6	+6.0
Formic acid, 0.5 per cent (after 17 weeks of 0.1 per cent)	6406	9	-0.66	-3.8	-29	-29.0

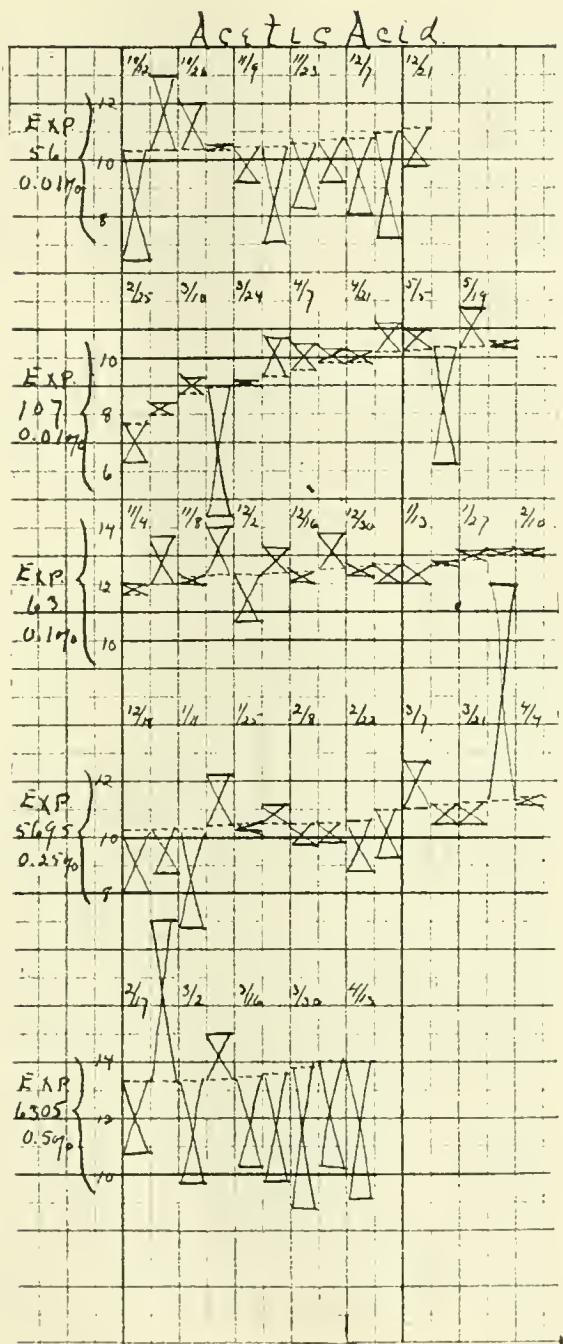


FIG. 4. ACETIC ACID ON FOOD CONSUMPTION

The numbers to the left represent grams of food consumed daily per rat. The numbers above each experiment are the dates of the observations. The dotted horizontal lines represent the standard food consumption; and the solid horizontal lines, joined to the dotted lines by crossed lines represent the actual food consumption.

food averages lies between -0.01 and $+1.4$ grams per rat per day, or -0.08 to $+19$ per cent.

The experiments with 0.1 per cent and 0.25 per cent acetic and formic acids arrange themselves in close proximity to both extremes; that is, the food consumption is normal; if anything, formic acid seems to stimulate appetite somewhat; but this is

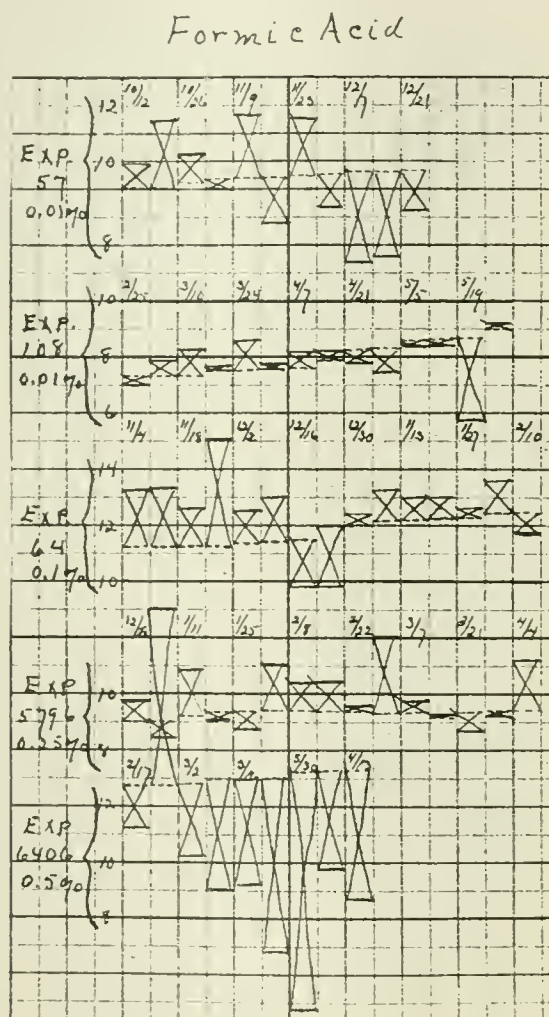


FIG. 5. FORMIC ACID ON FOOD CONSUMPTION

doubtless an accidental coincidence. With the 0.01 per cent acids, one experiment for each acid gave normal results, and the other a deficit; doubtless accidental, as explained under growth.

With the 0.5 per cent acids, there is serious interference with appetite; the food-consumption being reduced to 27 per cent for each acid.

The food and growth curves are in general very closely parallel. It is therefore impossible to say whether the failure of growth is the consequence of the failure of appetite, or vice versa.

MORTALITY

The data are shown in table 5. Briefly, no fatality occurred with either acid for concentrations of 0.5 per cent, equivalent to 0.36 cc. per kilogram of rat per day, for nine weeks, following after sixteen weeks of 0.1 per cent. In view of this, the occasional deaths from lower concentrations must be considered as accidental coincidences.

TABLE 5

Mortality

DRUG AND CONCENTRATION	DOSAGE	EXPERIMENT NUMBER	NUMBER OF ANIMALS	WEEKS OF FATALI- TIES	TOTAL DURA- TION	PER CENT OF FA- TALITIES
					<i>weeks</i>	
Acetic acid, 0.01 per cent.....	8.2	56	6	8	11	16
Acetic acid, 0.01 per cent.....	10.9	107	3	0	9	0
Acetic acid, 0.1 per cent.....	81.0	63	6	0	15	0
Acetic acid, 0.25 per cent (after 10 weeks of 0.01 per cent).....	210.0	5695	4	2, 4, 11	15	75
Acetic acid, 0.5 per cent (after 16 weeks of 0.1 per cent).....	360.0	6305	3	0	9	0
Formic acid, 0.01 per cent.....	8.2	57	6	11	11	16
Formic acid, 0.01 per cent.....	10.25	108	3	0	14	0
Formic acid, 0.1 per cent.....	90.0	64	6	0	15	0
Formic acid, 0.25 per cent (after 12 weeks of 0.01 per cent).....	160.0	5796	4	2	15	25
Formic acid, 0.5 per cent (after 17 weeks of 0.1 per cent).....	360.0	6406	3	0	9	0

CONCLUSIONS

Acetic and formic acids behave approximately quantitatively alike, when added to the drinking water of rats, in concentrations up to 0.5 per cent and daily doses up to 0.36 cc. of absolute acid per kilogram of body weight, for two to four months; this being the sole source of fluid for the animals.

Concentrations of 0.01 per cent to 0.25 per cent, corresponding to daily dosage of 0.2 cc. of acid per kilogram of body weight, produced no effect on growth, appetite, or consumption of fluid. They are therefore quite harmless.

The diuresis described in the literature for formates and acetates must be due to the sodium, potassium or lithium, and not to the formic radicle.

With concentrations of 0.5 per cent and daily dosage of 0.36 cc. of absolute acid per kilogram of rat, the appetite and growth but not the fluid consumption are materially, immediately and progressively diminished with both acids. This is evidently due to their acidity; but the experiments do not throw any light as to whether this is due to local action on digestion, or whether to some more profound disturbance of the acid-base equilibrium.

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THE EFFECT OF MORPHINE UPON THE ALKALI RESERVE OF THE BLOOD OF MAN AND CERTAIN ANIMALS

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Morphine, probably one of the most elaborately studied of the common pharmacopeial drugs used by the medical profession still possesses undiscovered actions awaiting for their unfolding the development of finer technic and new methods; actions which may eventually prove of some value in the treatment of certain diseases not influenced by mere narcosis. In certain respects morphine especially in its narcotic action behaves differently upon different animal species, the exact explanation for which is still lacking, so that it is advisable in studies with this drug to extend the observations to at least a number of different varieties of animals.

The newer methods for the accurate determination of the alkali reserve of the blood by Van Slyke and his coworkers and the observations of Underhill, Blatherwick and Goldschmidt (1) who found that morphine administered in small doses to fasting dogs resulted in the excretion of a strongly alkaline urine while fasted rabbits given even much larger doses of morphine revealed no such effect, seeming to accord with the well known resistance of rabbits to morphine narcosis, also made extended observations desirable. Only one experimental study was found in the literature on the effect of morphine upon the alkali reserve of the blood plasma and this study had been made by Hjort and Taylor (2) on dogs. They found that morphine in the form of sulphate administered subcutaneously to normal dogs in doses of 10 mgm. per kilo caused an increase in the alkali reserve which

is maintained at a high level for some hours. A repetition of the morphine injections after eight hours caused no further increase. In man, Collip (3) found that sleep produced negligible changes in the alkali reserve of the plasma although an increase was found by Hjort and Taylor in dogs after the administration of morphine subcutaneously. In an interesting recent investigation Barbour and Maurer (4) found in unanaesthetized rats that 7 to 8 per cent carbon dioxide breathing increases the respiratory volume by about one-third, the rate remaining practically unchanged, while morphine diminishes the respiratory volume by about three-fifths of the normal; the rate showing a smaller increase. Five or more hours are required for a return to normal conditions. Carbon dioxide (7 to 8 per cent) breathing was found to have practically no stimulating effect upon morphinized rats.

The investigations to be reported were carried on while trying to find a possible explanation for certain experimental therapeutic results obtained with morphine in tuberculosis. The species studied were man, sheep, dog and rabbit.

PROCEDURES

The variety of animals used precluded the possibility of using a single procedure without material harm to the animals, for obtaining the blood from all of them. The sheep were bled from the jugular vein directly into a clean dry centrifuge tube containing oxalate and paraffine oil as recommended by Van Slyke and Cullen (5) for collecting blood for the determination of plasma bicarbonate. In dogs and rabbits bleeding from the jugular or ear veins was found impractical while bleeding from the heart was hazardous usually resulting fatally after repeated bleedings so that a simple alternative method of bleeding from the ear was devised (6). The ear of the animal was shaved, thoroughly washed with soap, water and alcohol, finally being dried with a sterile sponge. The tip of the ear was cut off by a swift stroke of a sterile razor and the ear was immediately immersed into a 100 cc. beaker containing 30 cc. of pure paraffine

oil and a small amount of powdered potassium oxalate, the cut edge of the ear being placed in contact with the oxalate crystals. It was found that in this manner 10 to 20 cc. of blood could easily be collected which yielded a clear transparent plasma. The blood and paraffine oil were then carefully and without too much agitation transferred to a centrifuge tube and the usual technique followed. Subsequent bleedings from the same animals were made by merely removing the clot from the ear which had formed after the previous bleeding. This procedure caused

TABLE 1

Barometric corrections for high altitudes.

BAROMETER	$\frac{B}{760}$	BAROMETER	$\frac{B}{760}$
614	0.807	634	0.834
616	0.810	636	0.836
618	0.813	638	0.839
620	0.815	640	0.842
622	0.818	642	0.844
624	0.821	644	0.847
626	0.823	646	0.849
628	0.826	648	0.852
630	0.828	650	0.855
632	0.831		

the animal very little inconvenience and was less apt to cause the amount of excitement occasioned by bleeding from the jugular vein.

After the collection of a good clear untinted plasma, the approximate pH was determined by the direct method of Bayliss (7) substituting, however, phenolsulphonephthalein (using 0.2 cc. of a 0.01 per cent solution in 50 per cent alcohol to 1 cc. of plasma tested) for the neutral red indicator and using the comparator block of Levy, Rowntree and Marriott (8) with the Sørensen standards given by them and made of the primary potassium phosphate and secondary sodium phosphate solutions. The standard pH solution plus the indicator, and plasma without the indicator was compared with the plasma to which

the indicator had been added directly, and a control water tube. The CO₂ content was determined by the Van Slyke method. In correcting the observed carbon dioxide to 0°C. and 760 mm. pressure it was found that the range published by Van Slyke and Cullen was not suited for the Rocky Mountain districts but only was available for approximate sea level districts. The ratio $\frac{\text{barometer}}{760}$ was, therefore, computed for the higher altitude regions for convenience in correcting the CO₂ readings to a barometric pressure of 760 mm. The temperature corrections were made from Van Slyke and Cullen's tables.

TABLE 2

The effect of morphine upon the alkali reserve and pH of the blood plasma in dogs

DOG I					DOG II				
Time of bleed- ing	pH*	CO ₂ at 0°C. and 760 mm.†		Condition of the animal.	Time of bleed- ing	pH*	CO ₂ at 0°C. and 760 mm.†		Condition of the animal
		1	2				1	2	
Observations before the administration of morphine									
<i>hours</i>					<i>hours</i>				
0	7.2	45.7	43.8	Quiet	0	7.2	46.6	48.5	Quiet
2	7.1	40.9	40.9	Excited	2	7.1	48.5	46.6	Quiet
4	7.2	43.8	45.7	Quiet	4	7.2	48.6	49.5	Quiet
6	7.2	45.7	46.6	Quiet	6	7.1	46.6	46.6	Quiet
					8	7.2	48.6	46.6	Quiet
Morphine sulphate grain 1 given subcutaneously									
1	7.4	64.5	64.5	Very drowsy	1	7.2	50.4	51.3	Drowsy
3	7.4	64.5	65.6	Very drowsy	3	7.2	53.2	55.1	Drowsy
5	7.4	65.5	67.3	Very drowsy	5	7.3	56.0	57.9	Very drowsy
7	7.4	64.5	65.5	Very drowsy	7	7.3	64.5	65.5	Very drowsy
9	7.4	64.5	64.5	Very drowsy	9	7.4	64.5	65.5	Very drowsy
11	7.4	64.5	64.5	Drowsy	11	7.3	57.9	59.8	Drowsy
13	7.4	62.6	62.6	Drowsy	24	7.0	46.6	48.5	Quiet
15	7.3	60.7	59.8	Awake and quiet					
24	7.2	46.6	45.7	Awake and quiet					

* This is the average of two readings.
† Two readings are recorded on each plasma specimen.

The observations made on the dogs, were made on two mature male dogs, one shepherd dog, weighing 30 pounds, the other, a mastiff, weighing about 35 pounds. To obtain the normal ranges of these animals their blood plasma was examined the day before the administration of morphine. The day of the morphine test the examinations were carried over an interval until the plasma carbonates had returned to approximate normal figures. The results obtained on the dogs are given in table 2.

TABLE 3

The effect of morphine upon the alkali reserve and pH of the blood plasma in sheep

TIME OF BLEEDING	pH AVERAGE OF TWO READINGS	CO ₂ AT 0°C. AND 760 MM.		CONDITION OF THE ANIMAL
		1	2	
Observations before the administration of morphine				
<i>hours</i>				
0	7.2	48.5	49.4	Quiet
2	7.2	50.4	49.4	Quiet
4	7.1	49.4	49.4	Quiet
6	7.2	49.4	50.4	Quiet
Morphine sulphate grain 1 subcutaneously				
1	7.2	53.2	53.2	Quiet
3	7.4	56.0	57.9	Quiet
5	7.3	58.9	60.7	Quiet
7	7.2	53.2	54.1	Quiet
9	7.2	56.0	57.9	Quiet
11	7.3	49.4	49.4	Quiet
13	7.2	51.3	49.4	Quiet
15	7.2	49.4	51.3	Quiet

In dogs excitement decreases both the alkali reserve and pH of the plasma, while the administration of morphine causes a distinct increase in the alkali reserve and also an appreciable increase in the pH value of the plasma. This change persisting for some hours (eleven to fifteen) after the administration of the morphine.

The experiments on the sheep were performed on one adult sheep weighing 60 pounds, given a single administration of

morphine and one lamb weighing 25 pounds given several increasing amounts of the drug.

An examination of tables 3 and 4 reveals that the natural alkali reserve of the plasma of the lamb is distinctly lower than that for the sheep. Morphine administered subcutaneously,

TABLE 4

The effect of morphine upon the alkali reserve and pH of the blood plasma in the lamb

TIME OF BLEEDING	pH AVERAGE OF TWO READINGS	CO ₂ AT 0°C. AND 760 MM.		CONDITION OF THE ANIMAL
		1	2	
Observations before the administration of morphine				
<i>hours</i>				
0	7.3	39.0	39.0	Quiet
2	7.2	39.0	39.0	Quiet
4	7.1	32.4	33.4	Excited
6	7.2	39.0	39.0	Quiet
8	7.3	40.9	41.9	Quiet
Morphine sulphate grain $\frac{1}{2}$ subcutaneously				
1	7.3	46.6	48.5	Quiet
3	7.2	48.5	46.6	Quiet
5	7.4	51.3	51.3	Quiet
7	7.3	50.4	50.4	Quiet
Morphine sulphate grain 1 subcutaneously				
1	7.1	43.8	43.8	Quiet
3	7.1	39.0	39.0	Quiet
5	7.2	39.0	39.0	Quiet
Morphine sulphate grains 2 subcutaneously				
1	7.1	40.0	40.9	Quiet
3	7.1	39.0	39.0	Quiet

however, has the same effect in both, increasing the alkali reserve, although repeated injections in the lamb does not increase the effect over the primary injection.

The experiments on the rabbits were performed on two female rabbits, one weighing 12 pounds and the other 6 pounds. They were given a single injection of morphine. None of the animals

used for experimentation were fasted before the tests. The results on the rabbits are given in table 5. An examination of table 5 reveals a much more striking effect of the morphine upon the alkali reserve of the blood plasma in the rabbit than that produced in the dogs and sheep, whether the excited condition of the rabbits before and after the morphine effects had passed may not have aided in producing the greater difference is open to consideration.

TABLE 5

The effect of morphine upon the alkali reserve and pH of the blood plasma in rabbits

RABBIT 1					RABBIT 2				
Time of bleed-ing	pH	CO ₂ at 0°C. and 760 mm.		Condition of the animal	Time of bleed-ing	pH	CO ₂ at 0°C. and 760 mm.		Condition of the animal
		1	2				1	2	
Observations before the administration of morphine									
<i>hours</i>					<i>hours</i>				
0	7.0	39.0	37.2	Excited	0	6.9	34.3	34.3	Excited and struggling
2	7.1	37.2	37.2	Excited	2	6.9	34.3	36.2	Excited and struggling
4	7.2	39.0	39.0	Excited	4	7.0	33.4	34.3	Excited and struggling
6	7.2	39.0	40.9	Excited	6	6.9	34.3	36.2	Excited and struggling
Morphine sulphate grain $\frac{1}{4}$ given subcutaneously									
1	7.4	62.6	66.4	Quiet	1	7.3	70.2	71.1	Quiet
3	7.3	70.2	71.1	Quiet	3	7.3	71.1	71.1	Quiet
5	7.2	64.5	65.5	Quiet	5	7.1	60.7	59.8	Quiet
7	7.3	56.0	57.0	Quiet	7	7.2	51.3	53.2	Quiet
9	7.2	41.9	40.9	Excited	9	7.1	41.9	40.9	Excited
11	7.1	40.9	39.0	Excited	11	7.0	39.0	39.0	Excited

The experiments on man were performed on four adult males who were given from $\frac{1}{4}$ to $\frac{1}{2}$ grain of morphine sulphate subcutaneously.¹ Two of the men revealed a toxic as distinguished from a therapeutic reaction after this dose. They are grouped separately. The subjects were bled from the median basilic

¹ The author wishes to take opportunity here of expressing his gratitude to the men who voluntarily submitted to these experiments. When volunteers were called for, a generous response was found among the medical staff. Four men who appeared best suited for the tests were selected.

vein. No special precautions in diet were taken but the subjects were kept at rest and under careful observation during the entire tests. It is also to be noted that these observations in man cover not in each case but in total, all the intervals up to twenty-four hours. An examination of table 6 reveals no appreciable effect of morphine ($\frac{1}{4}$ and $\frac{3}{8}$ grains) in normal man given subcutaneously upon the alkali reserve of the blood plasma or upon the pH value of the plasma.

TABLE 6

The effect of morphine upon the alkali reserve and pH of the blood plasma; therapeutic dose

I. (196 POUNDS, WELL DEVELOPED)						II. (160 POUNDS, WELL DEVELOPED)					
Time of bleeding	pH	CO ₂ at 0°C. and 760 mm.		Pulse	Condition of man	Time of bleeding	pH	CO ₂ at 0°C. and 760 mm.		Pulse	Condition of man
		1	2					1	2		
Observations before the administration of morphine											
<i>hours</i>						<i>hours</i>					
0	7.1	65.5	62.2	78	Quiet	0	7.1	63.6	65.5	78	Quiet
2	7.1	67.2	65.5	80	Quiet	2	7.1	65.5	65.5	76	Quiet
4	7.1	65.5	65.5	84	Quiet	4	7.1	65.5	63.6	78	Quiet
6	7.1	66.4	65.5	80	Quiet	6	7.1	67.3	65.5	74	Quiet
Morphine sulphate given subcutaneously											
Grain $\frac{1}{4}$						Grain $\frac{3}{8}$					
1	7.1	67.2	67.2	78	Drowsy	12	7.2	67.3	65.5	78	Drowsy
3	7.1	65.5	67.2	74	Drowsy	15	7.1	63.6	65.5	80	Drowsy
6	7.1	65.5	65.5	80	Quiet	18	7.2	65.5	67.3	78	Quiet
9	7.1	65.5	65.2	78	Quiet	21	7.1	67.3	68.3	76	Quiet
						24	7.1	65.5	67.3	78	Quiet

The two subjects recorded in table 7 showed signs of acute morphinism. They did not reveal any appreciable effect of the morphine upon the pH or alkali reserve of the blood plasma even though they presented distinct symptoms of acute morphinism. It is also to be noted that these studies applied over a period of twenty-four hours subsequent to the drug admin-

istration. While these observations have revealed negative results in man it is possible that further study may offer conditions suitable for the demonstration of an effect analagous to that obtained in animals.

TABLE 7

The effect of morphine upon the alkali reserve and pH of the blood plasma of men with slight acute morphinism

III. (160 POUNDS, WELL DEVELOPED)						IV. (140 POUNDS, WELL DEVELOPED)					
Time of bleeding	pH	CO ₂ at 0°C. and 760 mm.		Pulse	Condition of man	Time of bleeding	pH	CO ₂ at 0°C. and 760 mm.		Pulse	Condition of man
		1	2					1	2		
Observations before the administration of morphine											
<i>hours</i>						<i>hours</i>					
0	7.1	65.5	65.5	76	Quiet	0	7.1	67.2	67.2	72	Quiet
2	7.1	67.2	65.5	74	Quiet	2	7.1	68.3	67.2	72	Quiet
4	7.1	66.4	65.5	76	Quiet	4	7.1	67.2	65.5	74	Quiet
6	7.1	66.4	68.3	74	Quiet	6	7.1	67.2	67.2	74	Quiet
8	7.1	65.5	63.6	72	Quiet						
Morphine sulphate grain $\frac{1}{2}$ given subcutaneously											
8	7.2	65.5	66.4	54	Cyanotic	2	7.1	65.5	65.5	64	Nausea, vom-
					nausea,					60	iting,
10	7.1	70.2	70.2	54	clammy	4	7.1	65.5	67.2	60	clammy
12	7.1	71.0	70.2	54	sweat,	7	7.1	65.5	65.5	60	sweat, cya-
14	7.2	70.2	70.2	60	tingling	9	7.1	67.2	68.3	64	notic,
					sensation						drowsy
17	7.1	66.4	68.3	78	Quiet	24	7.1	72.1	74.0	72	Quiet

SUMMARY

Morphine administered subcutaneously in the form of sulphate to rabbits, dogs and sheep distinctly increased the alkali reserve of the blood plasma of these animals. Likewise there was a slight but distinct effect upon the pH of the blood plasma. Excitement in dogs and rabbits decreases the alkali reserve of the blood plasma.

The natural alkali reserve of the plasma of the lamb is distinctly lower than that of sheep, but morphine increases it in

both animals. Repeated short interval injections of morphine do not tend to have a greater effect than a single injection. The alkali reserve increase as a result of the injection of morphine is greater in the rabbit than in either dogs or sheep (whether excitement plays a part in this greater effect was not determined). In man given a therapeutic or slightly toxic dose, morphine did not have a marked effect when administered subcutaneously (in dose from $\frac{1}{4}$ to $\frac{1}{2}$ grain) upon the pH or the alkali reserve of the blood plasma.

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THE ACTION OF THE "EMMENAGOGUE OILS" ON THE HUMAN UTERUS

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Prochnow (1), von Kurdinowski (2) and Macht have shown that volatile oils inhibit the movements of the mammalian uterus. Various drugs have been tested on the excised human uterus by Ruebsamen and Kligermann (4), J. A. Gunn (5) and Lieb (6), but, so far as the writer knows, the action of volatile oils on the human uterus has not yet been determined.

METHOD

Sufficient material was obtained from two cases in which hysterectomy was performed for uterine disease. As soon as possible after removal, the uterus and Fallopian tubes were placed in cold Ringer's solution (Locke's formula), and removed to the laboratory.

Movements of the uterus and Fallopian tubes were recorded by Kehrer's method in an apparatus similar to that described by Dale and Laidlaw (7) and shown in figure 1. A glass cylinder, *A*, containing Ringer's solution, previously heated to 37°C., is kept at this temperature by being surrounded with a copper water-jacket, *B*, in which the temperature is maintained by the heat from a Bunsen burner, *D*, being conducted along the copper rod, *C*, to the water in the water-jacket. The Ringer's solution is supplied with a constant stream of oxygen through the glass tube *E*. To the end of this tube is fixed one end of the organ whose movements are to be recorded (shown at *F*). The other end of the organ is fastened to a lever, *G*, which records the movements on a rotating smoked drum.

For the movements of the Fallopian tube a portion about an inch and a half long was taken from the middle, the attachment to the uterus and the fimbriated end being cut off. One uterus was unsuitable for experiment, but from the other enough apparently healthy muscle could be obtained to give satisfactory results. Longitudinal pencil-shaped strips about $1\frac{1}{2}$ inches long were cut from the muscle and their movements recorded as described.

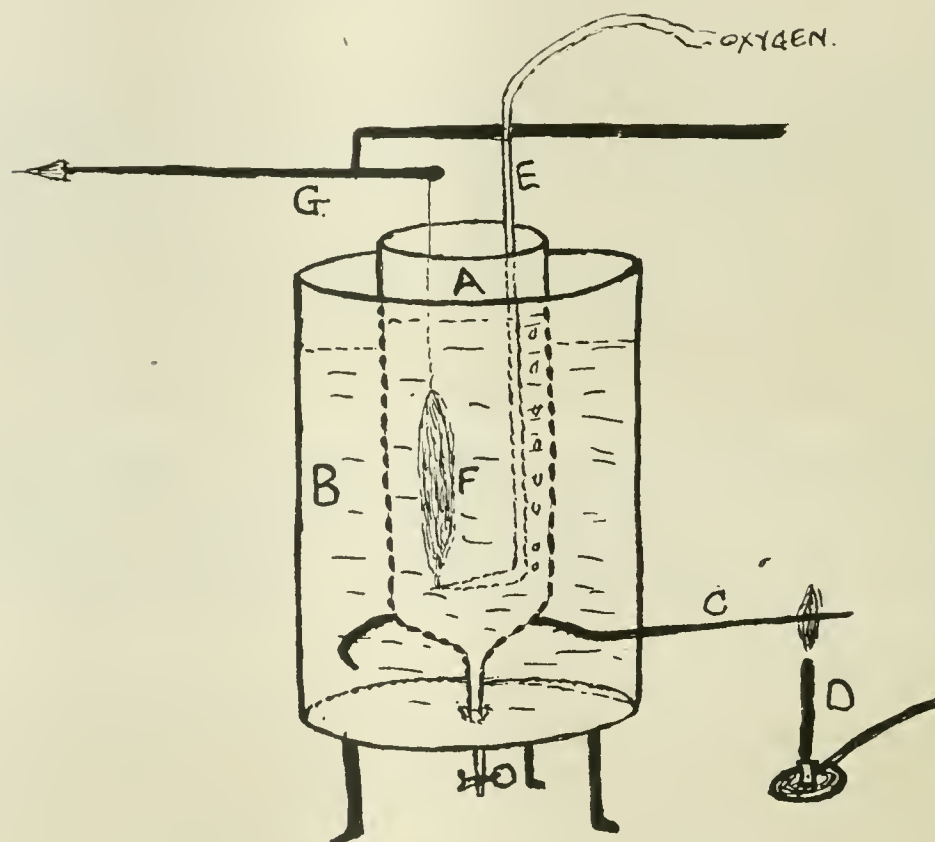


FIG. 1

The following drugs were used in the experiments: Oils of juniper, pennyroyal, rue, savin and tansy. A 1 per cent emulsion of each was made in Ringer's solution. After a normal tracing was taken, measured amounts of the emulsion were added to the Ringer's solution in the glass cylinder and the effects recorded.

RESULTS

1. *Fallopian tube*

Concentrations of the oils of 1 in 10,000 inhibited the movements of the Fallopian tube, and caused it to relax. Smaller concentrations were without effect. In no case was there any increase of movement.

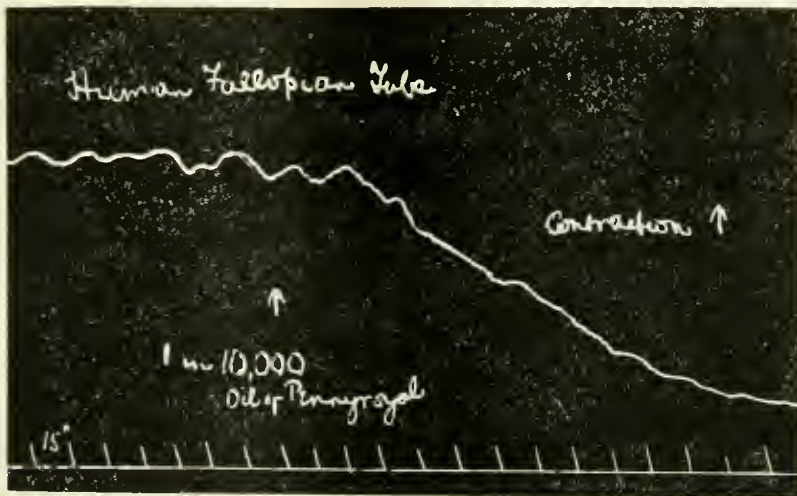


FIG. 2. THE EFFECT OF 1 IN 10,000 OIL OF PENNYROYAL ON THE MOVEMENTS OF THE HUMAN FALLOPIAN TUBE

Figure 2 shows the normal rhythmical movements of the human Fallopian tube and the relaxation and inhibition of movement produced on adding 1 in 10,000 oil of pennyroyal.

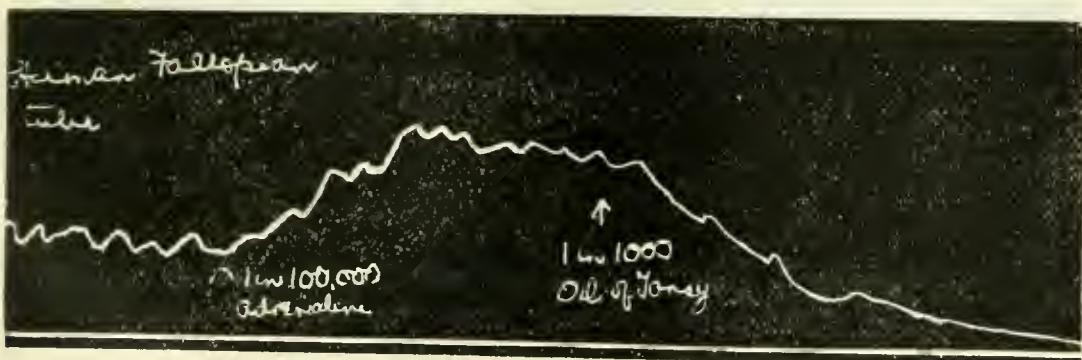


FIG. 3. THE EFFECT OF 1 IN 100,000 ADRENALINE AND SUBSEQUENT ADDITION OF 1 IN 1000 OIL OF TANSY ON THE MOVEMENTS OF THE HUMAN FALLOPIAN TUBE

Figure 3 shows the increase of tone after 1 in 100,000 adrenaline and this effect being antagonised on subsequent addition of 1

in 1000 oil of tansy, the movements quickly ceasing. The action of all the oils was similar but oil of pennyroyal seemed to be the most active.

2. *The uterus*

The action of these oils on the uterus was the same as on the Fallopian tubes but somewhat stronger solutions were necessary, marked loss of tone following the application of 1 in 3000 of the oils.

In figure 4 the effect of 1 in 2000 oil of savin is seen.

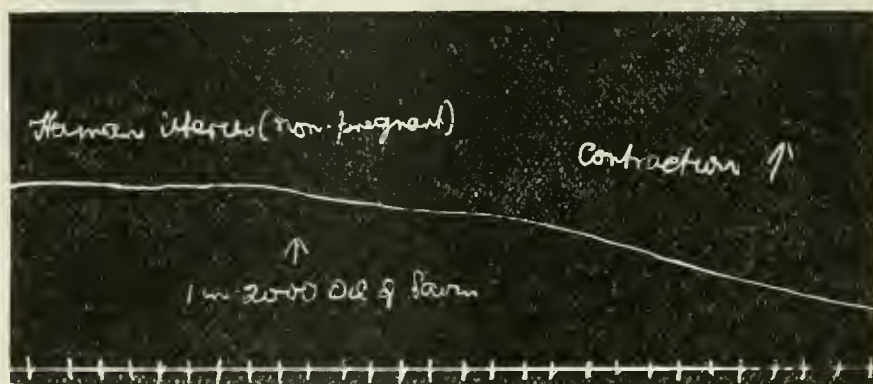


FIG. 4. THE EFFECT OF 1 IN 2000 OIL OF SAVIN ON THE TONE OF THE EXCISED HUMAN UTERUS

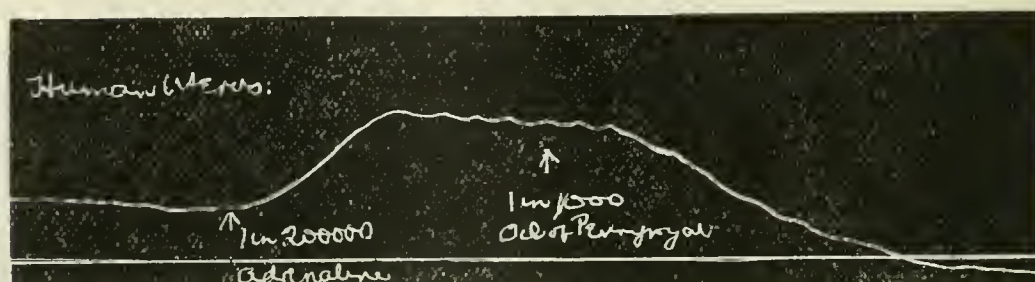


FIG. 5. THE EFFECT OF 1 IN 200,000 ADRENALINE AND SUBSEQUENT ADDITION OF 1 IN 1000 OIL OF PENNYROYAL ON THE HUMAN UTERUS

Figure 5 shows the effect of 1 in 200,000 adrenaline. It originated rhythmical movements in the quiescent uterus and caused an increase of tone. The subsequent addition of 1 in 1000 oil of pennyroyal quickly brought on paralysis of the uterus.

DISCUSSION

These experiments on the isolated human uterus and Fallopian tubes agree with the results of other observers on the excised uterus of other mammalia. It would seem that the emmenagogue oils in very small amounts have no action at all on the uterus. In higher concentrations such as could never be reached in the blood without producing dangerous, probably fatal, poisoning, they inhibit the uterine movements.

When abortion occurs after their use, it is probably an indirect result of the severe irritation and inflammation of the bowel and kidney. This may induce congestion and reflex movements of the uterus which may in some cases result in abortion.

The absence of specific stimulant action of these oils on the uterus renders them all the more dangerous poisons, as, after their failure in ordinary doses, large doses are sometimes taken, resulting in frequent poisoning, in a large proportion of cases without the production of abortion.

I am indebted to Dr. E. C. Crichton, Professor of Obstetrics and Gynaecology at the University of Cape Town for the materials for the experiments.

SUMMARY

The "emmenagogue oils" have no stimulant action on the human uterus and Fallopian tubes. In large doses they cause relaxation and inhibition of movement.

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ON THE INFLUENCE OF COLLOIDS ON THE ACTION OF NON-COLLOIDAL DRUGS. I

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From the Pharmaco-Therapeutical Institute, University of Leiden

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In a former communication (1) Le Heux and I have shown that the relation which exists between the concentration in which a drug is present and the effect it will produce, varies in proportion to the nature of the drug.

We found namely, that for drugs that are easily soluble in lipoids—and as a type of such we examined a number of fat soluble narcotics—the relation between concentration and action can be shown by a curve, such as appears in figure 1. In such a case the relation between concentration and action of the drug is constant; and in every phase of the curve an increase of concentration of 50 per cent will also produce an increase in action of 50 per cent.

For a number of drugs not soluble in lipoids but easily soluble in water, especially for the salts of the alkaloids and other basic poisons, a quite different proportion between concentration and effect was found—namely that shown in figure 2. In such a curve there is always a phase in which a small difference in the dose produces a great difference in effect and following this comes a phase in which a great difference in concentration results in a small difference in effect.¹

It is needless to say, that in looking for an explanation of this curious form of the curve shown in figure 2 we immediately

¹ Reid Hunt and Seidell. On studying the influence of thyreoidin on the sensitiveness of mice to acetonitril showed in 1909 that the curve which represents the relation between the dose of thyreoidin and its action (influence on the sensitiveness to acetonitril) has the form of a parabola, i.e., it belongs to type II.

thought of the possibility of an adsorption-phenomenon taking place, because, as is known, the curve that indicates the course of the adsorption of dyes by animal charcoal is entirely analogous

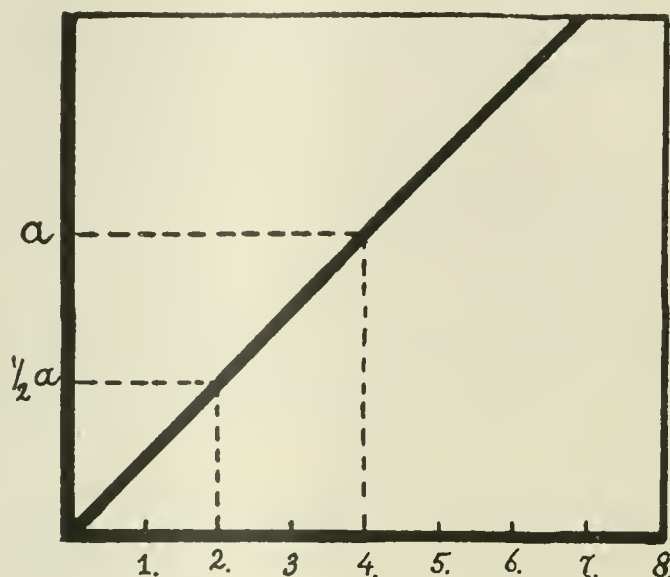


FIG. 1. CONCENTRATION-ACTION CURVE, TYPE I

Abcissus: Concentration of the drug (doses per kilogram animal). Ordinate: Action of the drug.

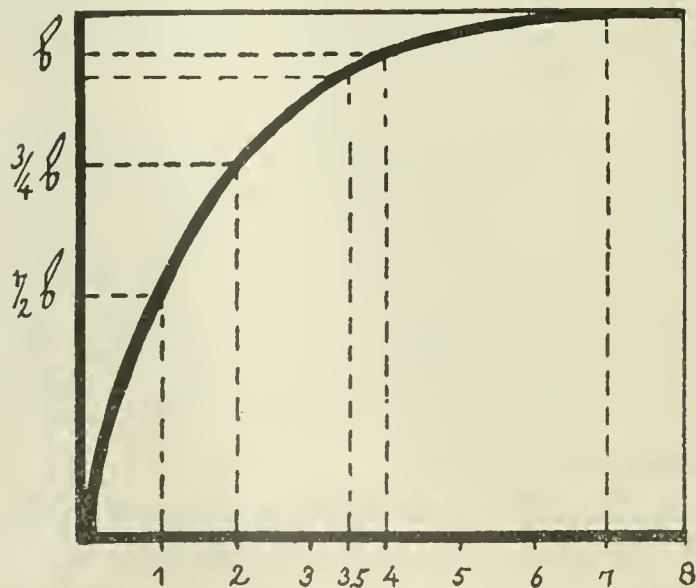


FIG. 2. CONCENTRATION-ACTION CURVE, TYPE II

to that shown in figure 2. To illustrate this we give in figure 3 a curve taken from Freundlich's book which indicates the proportion of the adsorption of benzoic acid and succinic acid by animal charcoal.

In order to find out if our supposition was correct, we first ascertained whether alkaloids and other basic poisons generally could be easily adsorbed by animal charcoal and whether if so they follow the same laws which prevail in the case of the adsorption of dyes by animal charcoal. This actually appeared to be the case—as is shown in figures 4 and 5 where the adsorption-isotherm of pilocarpine by animal charcoal and the adsorption-isotherm of nicotine by animal charcoal are given. The course of both curves is very similar to that of figure 2, but I need hardly say, that in order to accept in a special case the presence of an adsorptional phenomenon, it is not sufficient to have

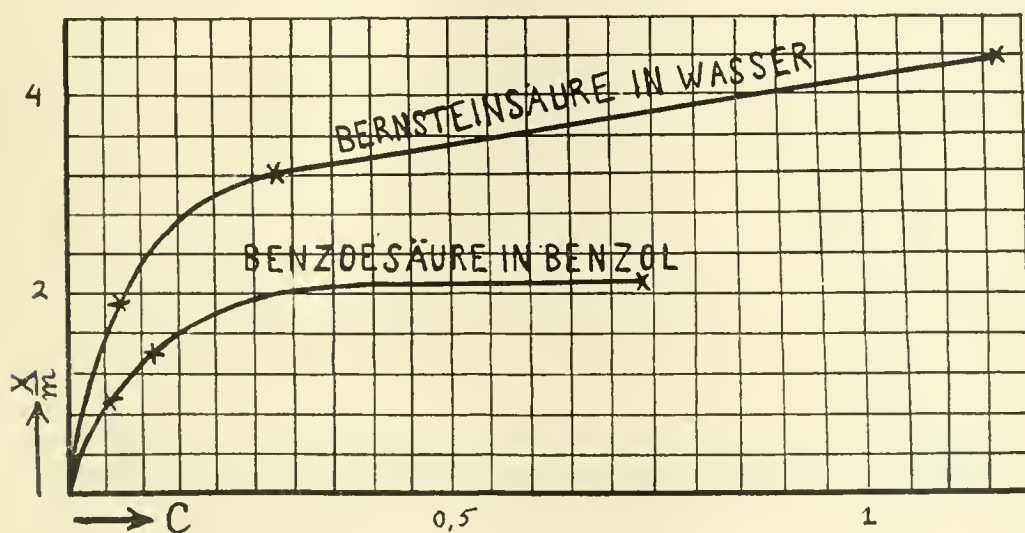


FIG. 3. ADSORPTION CURVES OF BENZOIC ACID AND SUCCINIC ACID
Taken from Freundlich Kapillarchemie

demonstrated that the curve has the course given in figure 2 or in figures 4 or 5. More conditions must be satisfied. One of these is, that a curve which indicates the relation between the logarithm of the concentration and the logarithm of the amount of the adsorbed material per unit of the adsorbens takes a straight line. We were able to prove that this condition was fulfilled in our case as will appear from figure 6. That we satisfied other conditions laid down by Freundlich (2, 3) for an adsorption is also shown in the publication mentioned above (1).

After it had thus appeared that alkaloids and other basic poisons are easily adsorbed by animal charcoal and that they

thereby followed such laws as prevail in the case of adsorption of dyes by animal charcoal, we endeavored to find out whether alkaloids are adsorbed by substances derived from animal tissues. Only when it is possible to prove this can the curious course of the curve of figure 2 be said to be due to an adsorption process.

When one examines in the simplest possible fashion the problem of the adsorption of alkaloids by tissue products, for

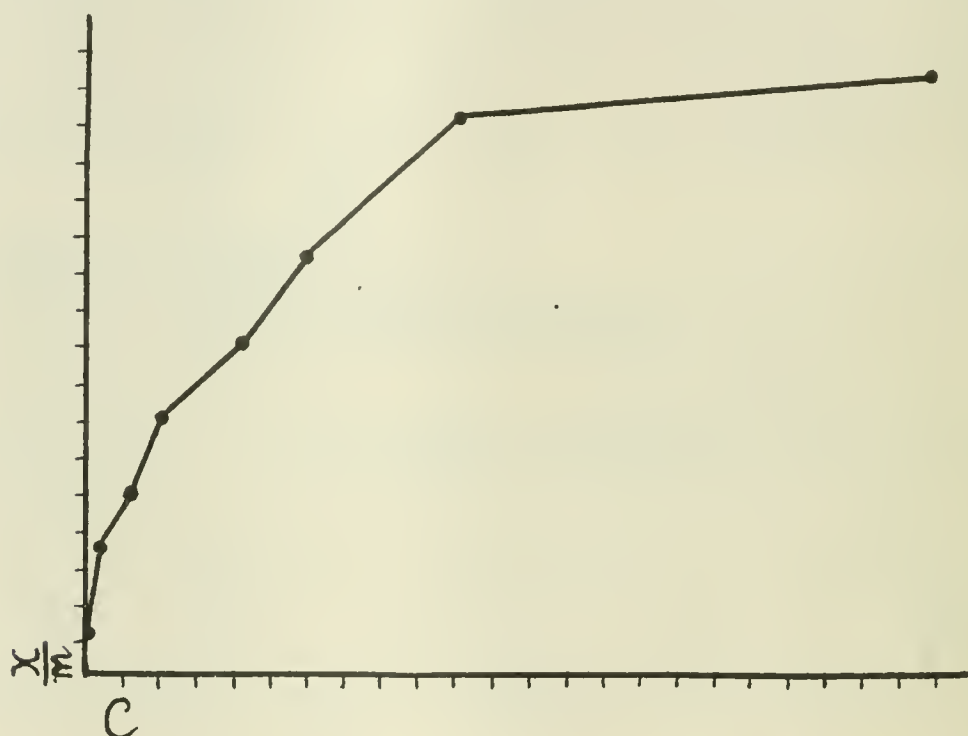


FIG. 4. ADSORPTION-ISOTHERM OF PILOCARPINE BY ANIMAL CHARCOAL

$\frac{H}{m}$ = Quantity of pilocarpine adsorbed by 100 mgm. of animal charcoal
 c = Concentration of pilocarpine hydrochloride in milligrams per liter

instance, when one examines whether an isolated organ in Tyrode solution is able to adsorb measurable amounts of a drug (as for example pilocarpine) it appears that no noticeable quantities of the alkaloid are being withdrawn from the solution by the excised organ.²

² This only holds good on the assumption that the doses of the drug are not higher than is necessary to produce a maximal effect and that the drug is not too long in contact with the isolated organ (not longer than five to ten minutes).

When for instance, in a vessel containing 75 cc. Tyrode solution was added a quantity of pilocarpine sufficient to produce a perceptible effect on a piece of excised gut it appeared that this

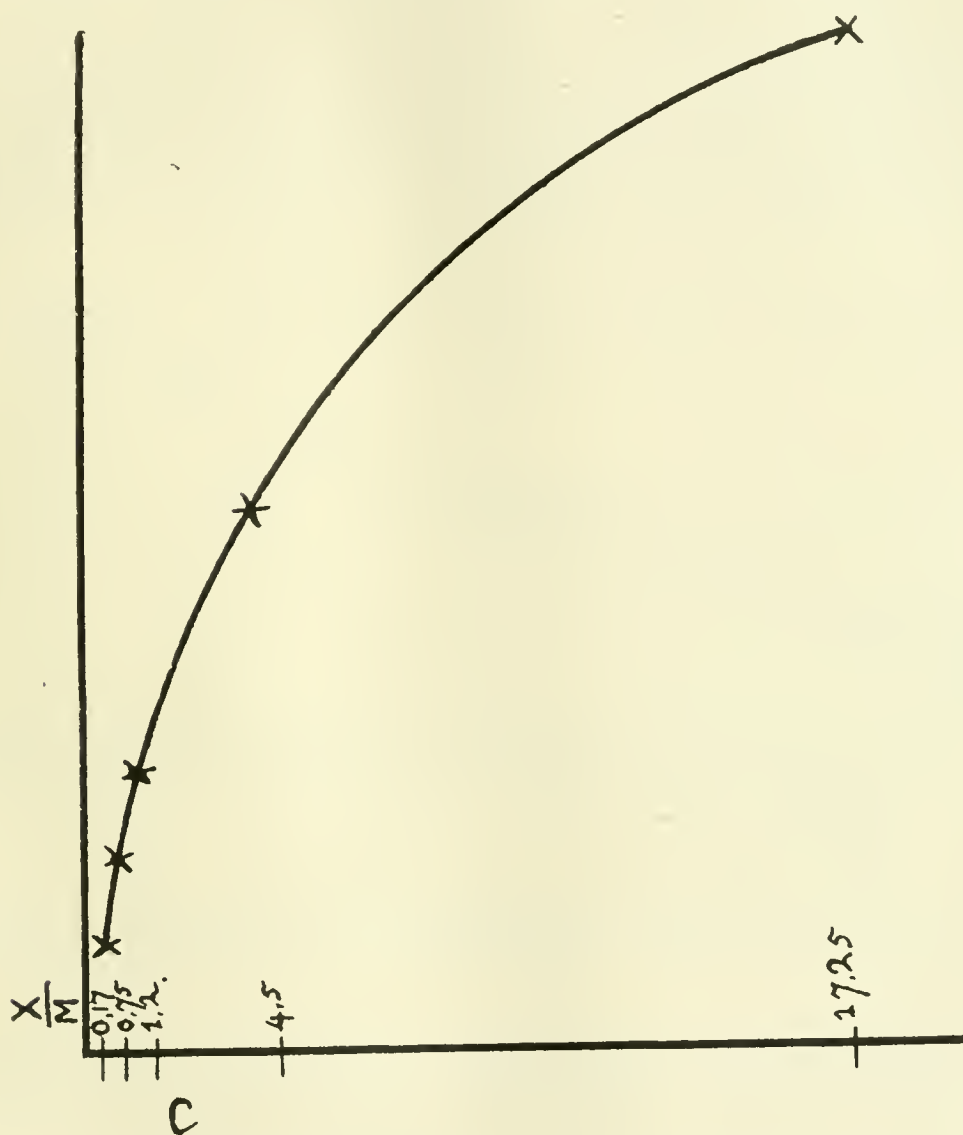


FIG. 5. ADSORPTION OF NICOTINE BY ANIMAL CHARCOAL

$\frac{H}{m}$ = Quantity of nicotine adsorbed by 100 mgm. of animal charcoal
 c = Concentration of nicotine in milligrams per liter

intestinal strip after having shown a maximum contraction as a result of the addition of the pilocarpine did not withdraw a perceptible quantity of the pilocarpine from the solution; for

when this intestinal strip was removed and thereupon a second strip was placed in the same solution it underwent precisely the same process as the first strip and it was found that this procedure could be repeated six or seven times without a perceptible weakening of the effect of the pilocarpine taking place. It appeared that this not only holds good for pilocarpine but also for atropine, which proves that during the action of these drugs no *perceptible* quantity of alkaloids was adsorbed by the intestine from the solution. This conclusion in no way proves that *in*

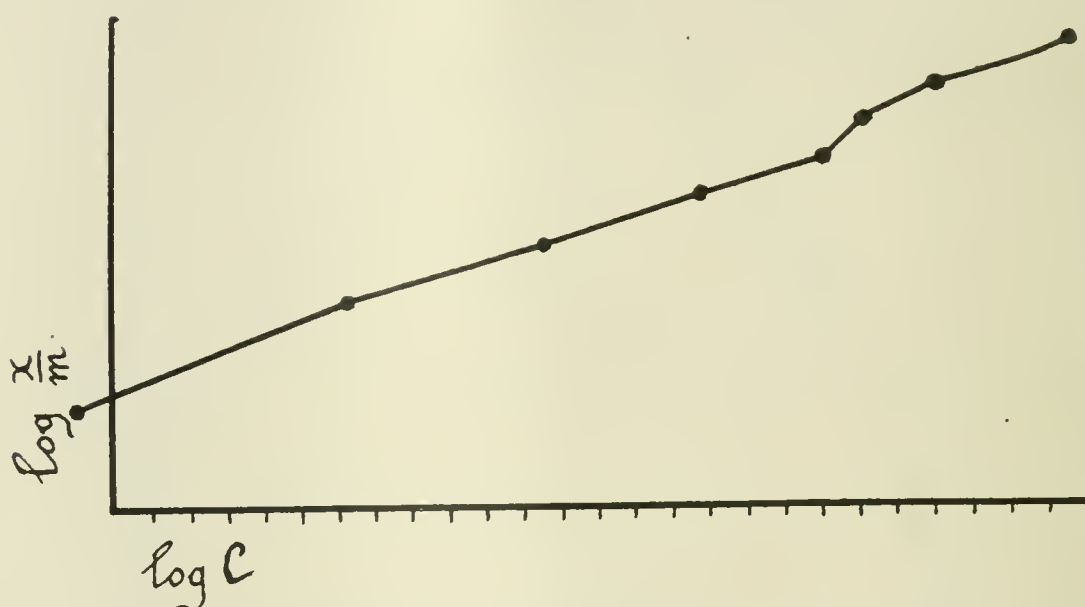


FIG. 6. ADSORPTION-ISOTHERM (LOGARITHMATED) OF PILOCARPINE BY ANIMAL CHARCOAL

very specific parts of the intestine an adsorption of alkaloids does not take place. Only when such an adsorption actually takes place—and we have in the communication referred to mentioned on what grounds we formed our opinion that this adsorption actually *does* take place—it must be an adsorption which in so far varies from that described by Freundlich, that it must be a very specific one,³ in other words, most tissue sub-

³ In a recent paper of Michaelis and Rona (Die Adsorbierbarkeit der oberflächenaktiven Stoffe durch verschiedene Adsorbentien sowie ein Versuch zur Systematik der Adsorptionserscheinungen. Biochem. Zeitschr., Vol. 102, p.

stances will adsorb no alkaloids, while such tissue substances on which alkaloids can have an effect, adsorb the alkaloids in a strong degree, and in a specific manner. Here I would at once mention, because in the course of the discussion which follows we shall need to know it, that we gave the name of *dominant chemoreceptors* to those parts of the tissue in which the alkaloids act specifically, and where they are according to our opinion specifically adsorbed. When it had become clear to us that alkaloids were adsorbed in a very specific way by the dominant chemoreceptors, at once the query arose whether receptors might be present in other parts of the body which also had a strong binding power for alkaloids (these we subsequently called secondary receptors).

In an other communication (4) it was proved by us that these secondary chemoreceptors are actually to be found on a large scale in the animal body. In seeking for this we were led by the following consideration: if it is true that alkaloids before they can act specifically on dominant chemoreceptors are adsorbed by them and when it is furthermore true that secondary chemoreceptors are also found in other parts of the body, then it must be accepted that the sensitiveness of an animal for a certain drug will not merely depend on the amount of the dose that is given and on the sensitiveness of the specific organs of the animal, but also on the proportion between the number of dominant and secondary chemoreceptors. If many dominant receptors are present, and few secondary ones, the animal will be very sensitive to a dose of a certain drug; if many secondary and few dominant receptors are present, the animal will be only slightly sensitive to that drug. We therefore realized that in order to find the secondary receptors we must choose an animal with a low degree of sensitiveness for a specific drug, because it might be possible that many secondary chemoreceptors were present. We actually succeeded in this. We chose to experi-

268, 1920), attention was drawn to the fact that the intensity of the adsorbing power of different adsorbentia is much more dependant on the chemical nature of the adsorbens than was thought before.

ment upon the rabbit, as an animal known to be only in a low degree sensitive to various alkaloids, among which atropine and pilocarpine. Our first experiments were with pilocarpine; we chose this drug because in the Pharmacological Institute in Utrecht where we were working at that time a method has been elaborated by which small quantities of pilocarpine can be determined with great accuracy by means of a physiological method. This is done by taking as a criterium for a pilocarpine action the effect of this drug on strips of the excised intestine of the cat suspended in Tyrode solution. In using this technique it was easy to show that rabbit-serum (and also liver extracts of the rabbit) actually contain substances which have a strong binding power for pilocarpine as can be shown in figure 7, *a-c*. In figure 7, *a*, 0.1 mgm. of pilocarpine hydrochloride is added to the Tyrode solution in which an isolated strip of cat-gut is suspended. In figure 7, *b*, the same quantity of pilocarpine is added to the same gut but this pilocarpine had been dissolved in rabbit-serum and has no influence on the gut. In figure 7, *c*, 2 mgm. of pilocarpine dissolved in rabbit serum are added and the contraction which ensues is smaller than that caused by 0.1 mgm. of pilocarpine in water, which proves that in this experiment more than $\frac{1}{20}$ of the pilocarpine had been made inactive by the rabbit-serum. In figure 7, *d*, 0.1 mgm. pilocarpine in water is again added to prove that the sensitivity of the gut had not been changed. In other experiments we found that very often a fortyfold quantity of pilocarpine dissolved in rabbit-serum had to be added to produce the same effect as a single dose of pilocarpine in water.⁴

It may be mentioned here that it is known that serum contains substances which per se are able to inhibit and also substances

⁴ Attention is called to the fact that not all the rabbit-sera tested had such a strong inhibiting power. Though a certain amount of inhibition was always demonstrable in every experiment we sometimes came across sera which proved to contain only a slight amount of inhibiting substances. In our first experiments which were made in Utrecht we found the sera to have *as a rule* a very strong inhibiting power; in the later experiments in Leiden this was different, the inhibition though never failing was as a rule much less marked. Experiments are planned to investigate this matter more thoroughly.



FIG. 7, a



FIG. 7, b



FIG. 7, c



FIG. 7, d

FIG. 7, a-d. STRIP OF CAT GUT SUSPENDED IN 75 CC. OF TYRODE SOLUTION

FIG. 7, a. 0.1 MGM. PILOCARPINE HYDROCHLORIDE (DISSOLVED IN WATER) GIVES MARKED CONTRACTION

FIG. 7, b. 0.1 MGM. PILOCARPINE DISSOLVED IN 0.1 CC. RABBIT SERUM GIVES NO CONTRACTION

FIG. 7, c. 2 MGM. PILOCARPINE DISSOLVED IN 2 CC. OF RABBIT SERUM GIVES CONTRACTION SMALLER THAN IN 7, a

FIG. 7, d. 0.1 MGM. OF PILOCARPINE DISSOLVED IN WATER (CONTROL)

In all the experiments of this sort the drug always is left in contact with the gut for three minutes and is then "washed out" by repeatedly bringing the gut in fresh Tyrode solution. The next dose of a drug was always given after an interval of from fifteen till twenty minutes.

which will increase the movements of the isolated gut but the quantities of serum we had to use in our experiments were always so small that the influence of these substances was negligible.

After having found that the action of pilocarpine could be inhibited by rabbit-serum the question at once arose whether this inhibition was due to a chemical destruction or to a physical binding. We could easily show that a chemical destruction certainly did *not* take place because if pilocarpine was added to rabbit-serum so that the action of the drug was greatly diminished we could by a very simple measure, i.e., extraction with alcohol or boiling with water regain all the pilocarpine in active form as is shown in figure 8, *a-e*.

FIG. 8, *a*FIG. 8, *b*FIG. 8, *c*FIG. 8, *d*FIG. 8, *e*

FIG. 8, *a-e*. ISOLATED STRIP OF CAT GUT SUSPENDED IN 75 CC. TYRODE SOLUTION

FIG. 8, *a*. ADDITION OF 0.1 MGM. PILOCARPINE HYDROCHLORIDE (DISSOLVED IN WATER) GIVES MARKED CONTRACTION

FIG. 8, *b*. ADDITION OF 1 MGM. PILOCARPINE DISSOLVED IN 1 CC. RABBIT SERUM GIVES ONLY SLIGHT CONTRACTION

FIG. 8, *c*. 0.1 MGM. PILOCARPINE (CONTROL)

FIG. 8, *d*. AN AMOUNT OF RABBIT SERUM AND PILOCARPINE, TREATED WITH ALCOHOL, EVAPORATED AND SO FORTH (SEE TEXT), CONTAINING, ON THE ASSUMPTION THAT ALL THE PILOCARPINE IS PRESENT IN ACTIVE FORM, 0.1 MGM. PILOCARPINE GIVES A CONTRACTION ONLY SLIGHTLY SMALLER THAN 0.1 MGM. PILOCARPINE IN THE CONTROL EXPERIMENT.

FIG. 8, *e*. 0.1 MGM. PILOCARPINE (CONTROL)

In figure 8, *a*, 0.1 mgm. of pilocarpine hydrochloride is added to an isolated strip of cat-gut suspended in 75 cc. Tyrode solution,

the drug is washed out and in figure 8, *b*, 1 mgm. of pilocarpine (10 times the first dose but mixed with rabbit serum) is given which exerts little action on the gut. In figure 8, *c*, again 0.1 mgm. of pilocarpine (dissolved in water) is given as a control to show that the sensitiveness of the gut has not been changed.

The stock solution of pilocarpine in rabbit-serum is now treated with alcohol, the alcohol is evaporated, the solution is filtered and water is added to make up the original volume. In figure 8, *d*, a quantity of this solution containing (on the assumption that all the alkaloid is regained in active form) 0.1 mgm. pilocarpine is given to the gut and this dose produces exactly the same effect as 0.1 mgm. of pilocarpine dissolved in water did before and afterwards (fig. 8, *e*).

Hereby it is proved that the pilocarpine is *not* destroyed but has been made inactive by a different process. It is not yet possible to *prove* definitely that this process is an adsorption but on the other hand it was shown that the binding of pilocarpine takes place in such a way that surface tension processes play an active rôle in it. Without going too much into detail we wish to refer to one series of experiments where we tried to find out the quantitative relation of this binding process. We therefore added to various samples of the same rabbit-serum different amounts of pilocarpine and determined how much of the pilocarpine was, after one or two hours, still present in an active form and by subtraction of the dose added in the beginning we could find the amount of pilocarpine that had been bound in every case. The result of one of these experiments is given in table 1.

TABLE 1

QUANTITY OF RABBIT-SERUM	AMOUNT OF PILOCARPINE (HYDROCHLORIDE) ADDED	CONCENTRATION OF PILOCARPINE STILL PRESENT IN ACTIVE FORM AFTER HAVING BEEN IN CONTACT WITH RABBIT- SERUM FOR 2 HOURS	QUANTITY OF PILOCAR- PINE (HYDROCHLORIDE) RENDERED INACTIVE (ADSORBED) BY THE RABBIT-SERUM
<i>cc.</i>	<i>mgm.</i>	<i>mgm. in 5 cc. serum</i>	<i>mgm.</i>
5	10	0.5	9.5
5	20	1.25	18.75
5	40	5.0	35.0
5	100	71.5	28.5

TABLE 2

QUANTITY OF RABBIT-SERUM	AMOUNT OF PILOCARPINE (HYDROCHLORIDE) ADDED	CONCENTRATION OF PILOCARPINE STILL PRESENT IN ACTIVE FORM AFTER HAVING BEEN IN CONTACT WITH RABBIT-SERUM FOR 2 HOURS	QUANTITY OF PILOCARPINE (HYDROCHLORIDE) RENDERED INACTIVE (ADSORBED) BY THE RABBIT-SERUM
cc.	mgm.	mgm. in 5 cc. serum	mgm.
5	5	0.33	4.67
5	10	0.4	9.6
5	20	2.5	17.5
5	40	25.0	15.0

TABLE 3

QUANTITY OF RABBIT-SERUM	AMOUNT OF PILOCARPINE (HYDROCHLORIDE) ADDED	CONCENTRATION OF PILOCARPINE STILL PRESENT IN ACTIVE FORM AFTER HAVING BEEN IN CONTACT WITH RABBIT-SERUM FOR 2 HOURS	QUANTITY OF PILOCARPINE (HYDROCHLORIDE) RENDERED INACTIVE (ADSORBED) BY THE RABBIT-SERUM
cc.	mgm.	mgm. in 5 cc. serum	mgm.
5	5	0.5	4.5
5	10	0.5	9.5
5	20	2.0	18.0
5	40	12.5	27.5
5	100	25.0	75.0

In figure 9 a curve is given constructed from the data of table 1 indicating the relation between the amount of pilocarpine adsorbed by a given quantum of serum and the concentration of the pilocarpine left in active form in the solution. It is clear that this curve is very similar to an adsorption-isotherm (Freundlich) but—as mentioned above—an actual proof that an adsorption is present in this case cannot be given, but on the other hand it has been shown definitely that there is *no* chemical destruction of the pilocarpine. We lay especial stress on this point because in our opinion a chemical destruction could never given an explanation of the relative insensitiveness of the rabbit for pilocarpine. This drug, when injected into the bloodstream acts very quickly, i.e., in a few minutes; and a chemical destruction would always come too late to prevent the *primary* toxic action of the drug. A physical binding (adsorption) can act very quickly, it can exert its maximum action within one or

two minutes and it therefore can protect the animal against toxic amounts of the drug and this holds good not only for the case of pilocarpine but also—as will be proved in a later communication—for atropine, cocaine and other drugs. We of course do not deny that chemical destruction of drugs plays a very large and very important part in the animal body (we refer to the beautiful experiments of Hatcher and Eggleston on the destruction of local anaesthetics in the body and especially

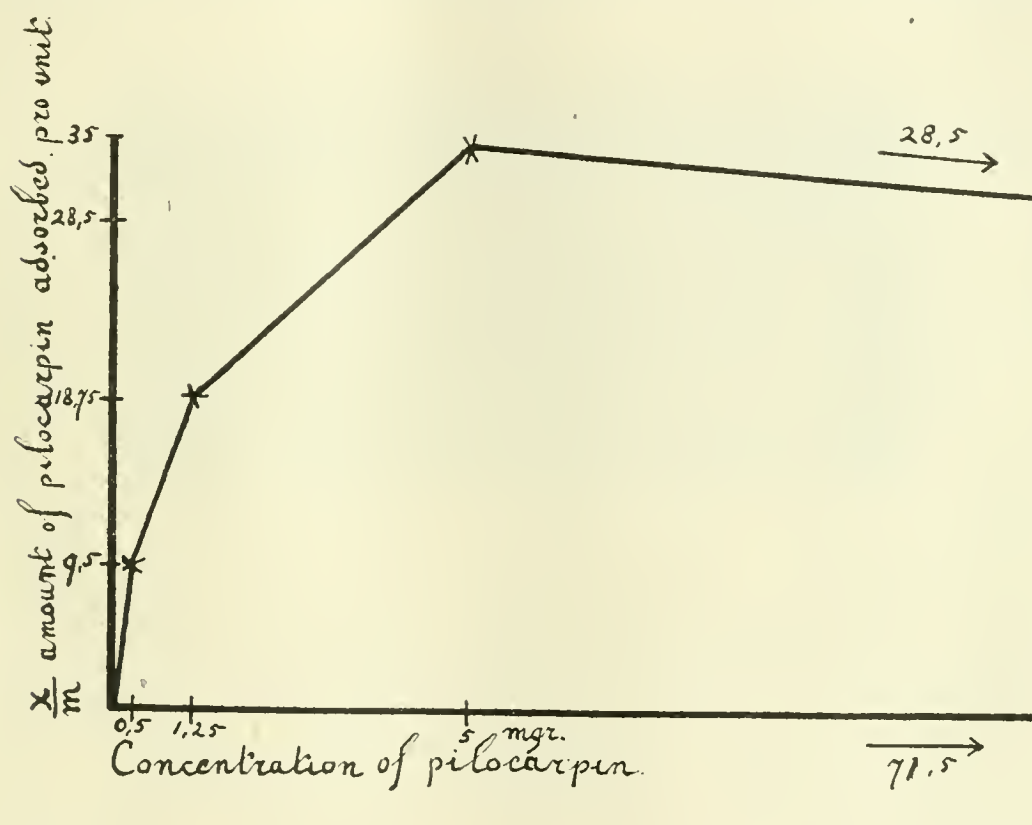


FIG. 9

in the liver) but we think that in cases where a drug has a very quick and acute toxic action the *primary* protection cannot be a chemical destruction but must be a different process; in our case we think to have proved that the binding power of the rabbit's blood for pilocarpine is an important factor in heightening the resistance of this animal for pilocarpine.

It goes without saying that we tried to find out the nature of the inhibiting substance in rabbit-serum. Our results in this

direction are so far rather poor. We could show, that lecithine, cholesterine and cerebrin⁵ had no inhibiting power, whereas albumoses (i.e., peptone Witte) had a slight adsorbing effect. Endeavours to isolate the adsorbing substance by the ordinary procedure as precipitation with ammonium sulfate and so on and by dialysis failed entirely.

It is necessary to call attention to the fact that Dixon and Hamill (5) already in 1909 stated that the inactivation of alkaloids by tissue extracts could not be a chemical destruction but must be a physical process. They stated also—without giving so far as I can find experimental proof for it—that by mere dilution with water the inhibiting power of tissue extracts on alkaloids can be abolished. This statement of Dixon and Hamill, later also expressed by Dixon and Ransom (6)—which I found after having published our first results in this field—is completely in accord with our experimental findings, only Dixon goes farther and states that this inhibition of the action of alkaloids by tissue and serum is only dependent on the “colloidal nature” of the liquid. This last statement cannot be confirmed by us. We found a very great difference in the adsorbing power of sera of different animals. The rabbit-serum usually adsorbs pilocarpine very powerfully, but cat-serum and cow-serum have this property only in a very slight degree, whereas dog-serum as a rule has no adsorbing power at all, as is shown in figure 10. In this experiment at first 0.2 mgm. of pilocarpine was given to a piece of cat-gut suspended in Tyrode solution, this dose of pilocarpine caused a marked contraction of the gut. After having “washed out” the effect of this dose, the same amount of pilocarpine was added, but this time the pilocarpine had been mixed in dog-serum and this gives exactly the same contraction of the gut as before. Subsequently another dose of pilocarpine dissolved in water is given to prove that the sensitiveness of the gut has not been changed during the experiment.

We therefore think—and experiments to be reported later on only confirmed this opinion—that the binding capacity of rabbit-

⁵ For providing us with a certain amount of very pure lecithine of cerebrin and of other substances we are greatly indebted to Dr. Levene, Rockefeller Institute.

serum cannot only depend on the colloidal nature of this serum (as it is impossible to assume that the colloidal nature of this serum differs so much from that of dog-serum) but must be due to the occurrence in this serum of substances which have a very specific adsorbing power for pilocarpine and probably also for other alkaloids.

After finishing the experiments reported above the question arose whether the adsorbing power of rabbit-serum for pilocarpine could also be demonstrated in vivo and how far the relative

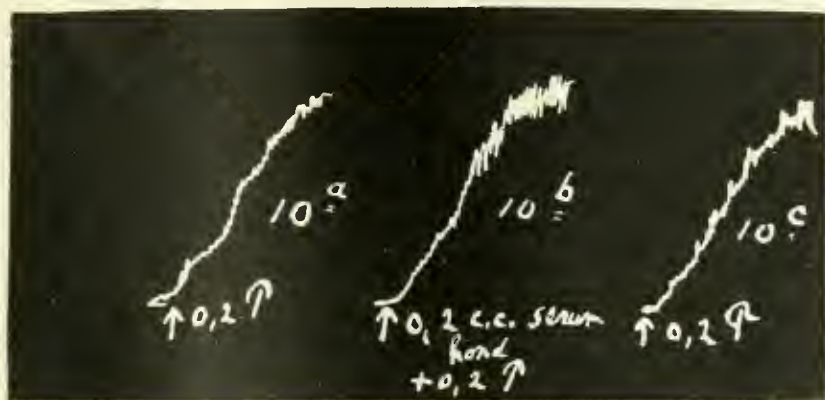


FIG. 10, a-c. ISOLATED CAT GUT SUSPENDED IN 75 CC. OF TYRODE SOLUTION

FIG. 10, a. ADDITION OF 0.2 MGM. OF PILOCARPINE HYDROCHLORIDE (FROM STOCK SOLUTION IN WATER) GIVES MARKED CONTRACTION

FIG. 10, b. 0.2 MGM. PILOCARPINE DISSOLVED IN 0.2 CC. DOG SERUM GIVES EXACTLY THE SAME CONTRACTION

FIG. 10, c. 0.2 MGM. PILOCARPINE AS CONTROL

insensitiveness of the rabbit for pilocarpine could depend on the presence of adsorbing substances in the serum of the animal.

In order to test this point it was first of all necessary to determine the lethal dose of pilocarpine for the rabbit and for another animal, i.e., the cat and we understood that in this case the poison had to be administered intravenously in a relatively short time.

Generally speaking the relative insensitiveness of an animal for a certain drug can be caused in the following ways.

1. The drug, when given per os is destructed in the alimentary canal or it is not absorbed by the gut.

2. The drug after having been absorbed by the gut is so rapidly destroyed in the body or is so rapidly excreted that it has no opportunity to act.

3. The different organs of the animal (heart, gut, central nervous-system, etc.) are not sensitive to the action of the drug; the dominant receptors for the drug are more or less completely absent.

4. The drug is resorbed by the gut but cannot exert an influence on the dominant receptors because there are a large amount of secondary receptors in the body which take so much of the drug, that little is left for the dominant receptors.

According to our assumption the last mentioned factor plays a large part in the case under discussion.

It is obvious that to rule out the first possibility (i.e., destruction in the gut or poor absorption) the pilocarpine had to be given intravenously; the second possibility can be ruled out as firstly, a chemical destruction of pilocarpine cannot take place within a few minutes and, secondly, pilocarpine is not in any noticeable degree excreted. The third possibility is an important one. Straub (7) has demonstrated recently that the insensitivity of the rat for strophantine is explained by the fact that the isolated heart of the animal is highly *insensitive* to strophantine there being a lack of dominant chemoreceptors. This assumption however does not hold good in our case as we know that although the rabbit is only slightly sensitive to pilocarpine and atropine; the isolated organs of this animal are very sensitive to these poisons, indeed v. Lidth de Jeude could prove (8) that the isolated gut of the rabbit is about ten times more sensitive to atropine than is the isolated gut of the cat.

From a theoretical point of view it was therefore very likely that the last mentioned possibility—a great amount of secondary receptors—would prove to be true, but to test this possibility it was necessary—as stated above—to determine the lethal dose of pilocarpine for the cat and the rabbit, the drug being injected in relatively large amounts so as to bring forth the acute poisoning effect of the drug and excluding the possibility of a chemical destruction in the animal body. According to this we performed the following experiments:

I. Cat, 2 kgm.; aethernarcosis, cannula in vena femoralis

- 2:20 Started infusion of 1 pro mille of pilocarpine hydrochloride in physiological salt solution, rate of infusion about 2 to 3 cc. per minute, after 3.5 cc. very marked secretion of saliva, pupils dilated, dyspnoe.
- 2:25 Infused; 10 mgm.; very marked secretion of saliva, pupils contracted, pulse rate about 100 per minute, infusion stopped.
- 2:30 Infusion started again.
- 2:50 50 cc. have been infused, marked dyspnoe.
- 3:15 125 cc. have been given: convulsions.
- 3:40 Respiration stops, heart still beating. Total amount of pilocarpine given 220 mgm. Lethal dose on intravenous infusion of diluted solution 110 mgm. per kgm.

II. Cat, 2.4 kgm.; aethernarcosis, cannula in vena femoralis

- 2:35 Started infusion of 1 per cent of pilocarpine hydrochloride in physiological salt solution, about 1 cc. per minute.
- 2:37 Secretion of saliva, pulse rate 120.
- 2:45 100 mgm. of pilocarpine have been infused; very marked secretion of saliva; pupils contracted.
- 2:55 Respiration stops; heart is still beating. Total amount of pilocarpine given: 220 mgm., 92 mgm. per kgm. Lethal dose on infusion of concentrated solution 92 mgm. per kgm.

III. Rabbit, 1.9 kgm.; aethernarcosis, cannula in vena femoralis

- 4:00 Started infusion of 1 per cent, pilocarpine hydrochloride in physiological salt solution.
- 4:08 8 cc. infused, marked salivation, pupils dilated.
- 4:10 10 cc. infused, defaecation, pupils smaller.
- 4:18 40 cc. infused, very marked salivation, pupils contracted.
- 4:27 70 cc. infused, dyspnoe.
- 4:55 120 cc. infused.
- 5:00-6:50 Larger amounts of pilocarpine are injected; first 1 per cent solution, later 1 per cent solution. At 6:50 total amount given: 800 mgm.; animal still living, experiment stopped. Lethal dose is above 420 mgm. per kgm.

IV. Rabbit, 1.7 kgm.; aethernarcosis, cannula vena femoralis

- 10:49 Started infusion of 1 per cent pilocarpine hydrochloride in physiological salt solution.
10:51 10 mgm. infused; salivation.
10:53 30 mgm. infused, marked salivation; defaecation.
11:30 500 mgm. infused; convulsions.
11:38 Respiration stops, heart still beating. Total amount given: 620 mgm. Lethal dose on infusion of concentrated pilocarpine solution 365 mgm. per kgm.

These experiments, though few in number suffice to prove that cats when tested in this way, i.e., by infusion of concentrated solutions of the drug—which causes acute death—are three to four times more sensitive to the action of pilocarpine than rabbits. We now tried to prove that the lethal dose for the cat could be enlarged by adding rabbit-serum to the pilocarpine. It was of course not possible to use the same technique as in the first experiments as the injections of larger doses of foreign serum into the cat is not an indifferent measure. We therefore proceeded in a different way. We knew from former experiments that a dose of 10 mgm. pilocarpine injected subcutaneously into a cat had a very strong effect on the secretion of saliva, strong excitation of the nervous system and so on and was fatal within twenty-four hours. We now injected three cats 10 mgm. of pilocarpine which had been dissolved previously in 10 cc. rabbit-serum. These animals showed only very slight symptoms and recovered completely. Here then it was proved that the rabbit-serum was able to protect the cat against a dose of pilocarpine which otherwise would certainly have been fatal.

In the course of these experiments we found that cat-serum could also inhibit the action of pilocarpine if mixtures of serum and pilocarpine were injected subcutaneously, however rabbit-serum acted more strongly in this respect than the cat-serum.

The following (shortened) protocol gives an instance of such an experiment.

TIME	CAT A	CAT B	CAT C
4:5	10 mgm. pilocarpine hydrochloride dissolved in 10 cc. of physiological salt solution per kilogram	10 mgm. pilocarpine hydrochloride mixed with 10 cc. of cat-serum per kilogram	10 mgm. pilocarpine hydrochloride mixed with 10 cc. of rabbit-serum per kilogram
4:7		Salivation (some drops)	
4:8	Vomiting movements, dyspnea		Very slight salivation (1 drop)
4:12	Salivation	Marked salivation	Salivation (a few drops and distinctly less than cat B) dyspnea
4:16	Strong salivation	Marked salivation	No salivation; dyspnea
4:18	Lies down, vomits; strong salivation; very ill	Marked salivation	No salivation; dyspnea
4:19	Ibid.	Ibid.; diarrhoea	Very slight salivation (1 drop)
4:26	Ibid.	No salivation; dyspnea	No salivation; dyspnea
4:35	Lies down, marked dyspnea, strong salivation	A few drops of saliva	No salivation
4:45	Ibid.	No salivation	Diarrhoea; 1 drop of saliva
4:50	Ibid.	A few drops of saliva	Looks normal, slight dyspnea
5:10	Ibid.	Looks normal, slight dyspnea; no salivation	Looks normal, slight dyspnea, 1 drop of saliva
5:12	Ibid.	Diarrhoea; vomits; dyspnea	Normal
9:00	Looks normal	Normal	Normal

In this case then 10 mgm. of pilocarpine in physiological salt solution caused very intense symptoms of pilocarpine poisoning. Cat A though looking normal at 9:00 p.m. was found dead in his cage the next morning. Cat B, which received 10 mgm. of pilocarpine per kilogram mixed with cat-serum also

showed marked symptoms of pilocarpine poisoning, though much less than cat A and he did *not* die. The symptoms of cat C which was injected with the same dose of pilocarpine per kilogram but mixed with rabbit-serum were much less marked as those of cat A. The difference in the reaction of cats B and C can be seen from the protocol but was much more clearly visible to the observer during the experiment.

In order to prevent mistakes we call attention to the fact that of course a physical binding—such as takes place in this case—can never protect the animal for *a long time*, if there is not a chemical process acting to destroy the poison gradually or if the poison is not excreted. However we think that the phenomenon of adsorption or binding is of primary importance since the chemical destruction can only be useful in a later stage, when the first acute effects of the drug have been inhibited in another way.

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EFFECT OF SOME ANTIPYRETICS ON THE BEHAVIOR OF RATS IN THE CIRCULAR MAZE

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INTRODUCTORY

In a preceding paper Macht and Mora (1) described the effect of opium alkaloids on the behavior of rats, as studied in the circular maze. Following that investigation it was interesting to inquire into what the effect would be on the behavior of rats when that other great class of analgesic drugs, namely, the coal tar antipyretics, are administered. Accordingly, the present research was undertaken. The following drugs were studied: Acetanilid, acetphenetidin, salol, sodium salicylate, antipyrine, pyramidon, and also quinin sulphate. In addition to these drugs, various combinations of them were also experimented with. All the drugs used in the experiments were administered either by hypodermic or intraperitoneal injection. The method of study was the same as in the case of the opium alkaloids. The behavior of the animals was observed in the circular maze. This apparatus has already been described, but for the sake of convenience a description of the same is again given in this place.

DESCRIPTION OF THE MAZE

The circular maze shown in figure 1 is made with wooden base and aluminium walls. The base is 150 cm. in diameter and 4 cm. in thickness. Its upper surface is marked off by grooves into a series of concentric circles. The diameter of each of the circles is as follows, beginning with the outermost one: 140 cm., 120 cm., 100 cm., 80 cm., 60 cm., 40 cm., and 20 cm. Into

the circular grooves are inserted sheets of aluminium 18.5 cm. high and 0.8 mm. thick. Each strip of aluminium is cut just 10 cm. shorter than the length of the circular groove into which it is to be fitted, thus giving an opening into the alley. By means of this arrangement it is possible to slide the aluminium around in its groove and thus to place the entrance in any desirable posi-

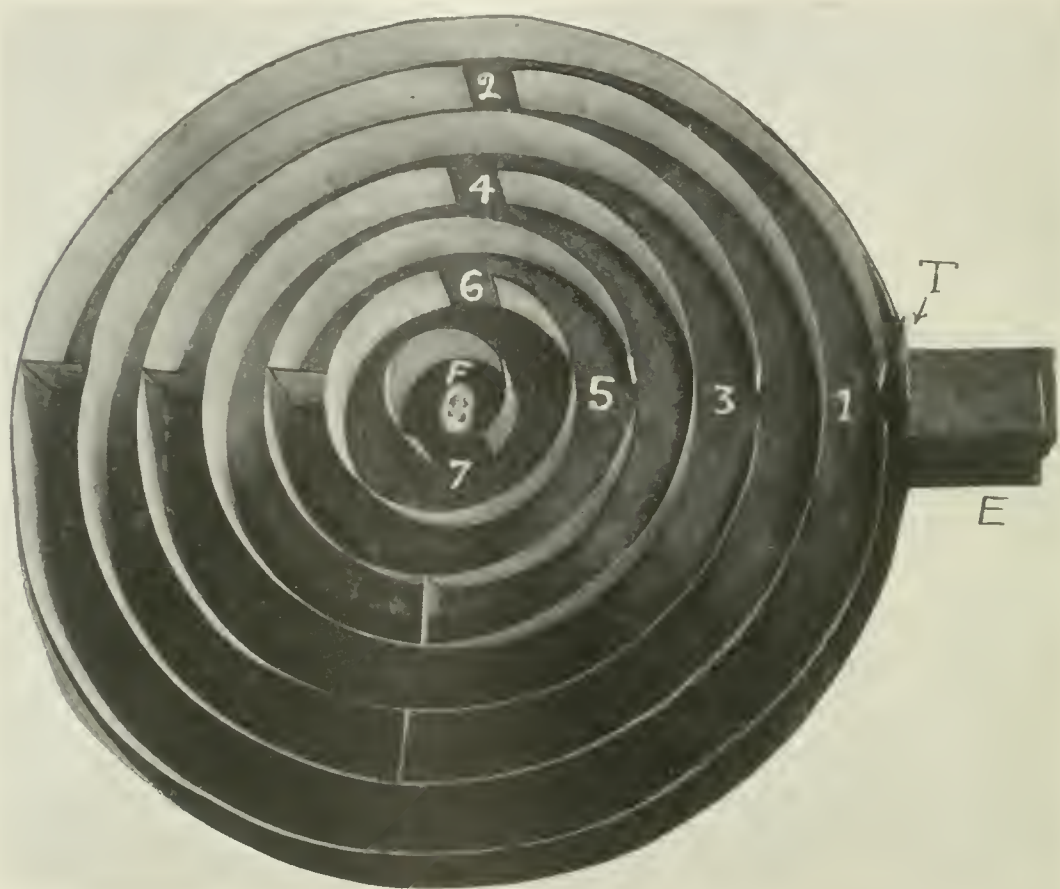


FIG. 1. CIRCULAR MAZE VIEWED FROM ABOVE

E, entrance cage; *T*, trap door leading into first alley; nos. 1 to 7, indicate the gates to the successive alleys; *F*, food.

tion. In the present investigation the openings or entrances to the alleys were placed in the position indicated in figure 1, there being seven openings so arranged that the rat had to make alternate turns to right and left, in the order indicated by nos. 1 to 7. In addition to the doors or openings, the alleys were also provided with obstructing partitions, which formed a number of blind cul-de-sacs.

The camera lucida attachment invented by Professor Watson is illustrated in figure 2. A large plate glass mirror, M , 91 cm.

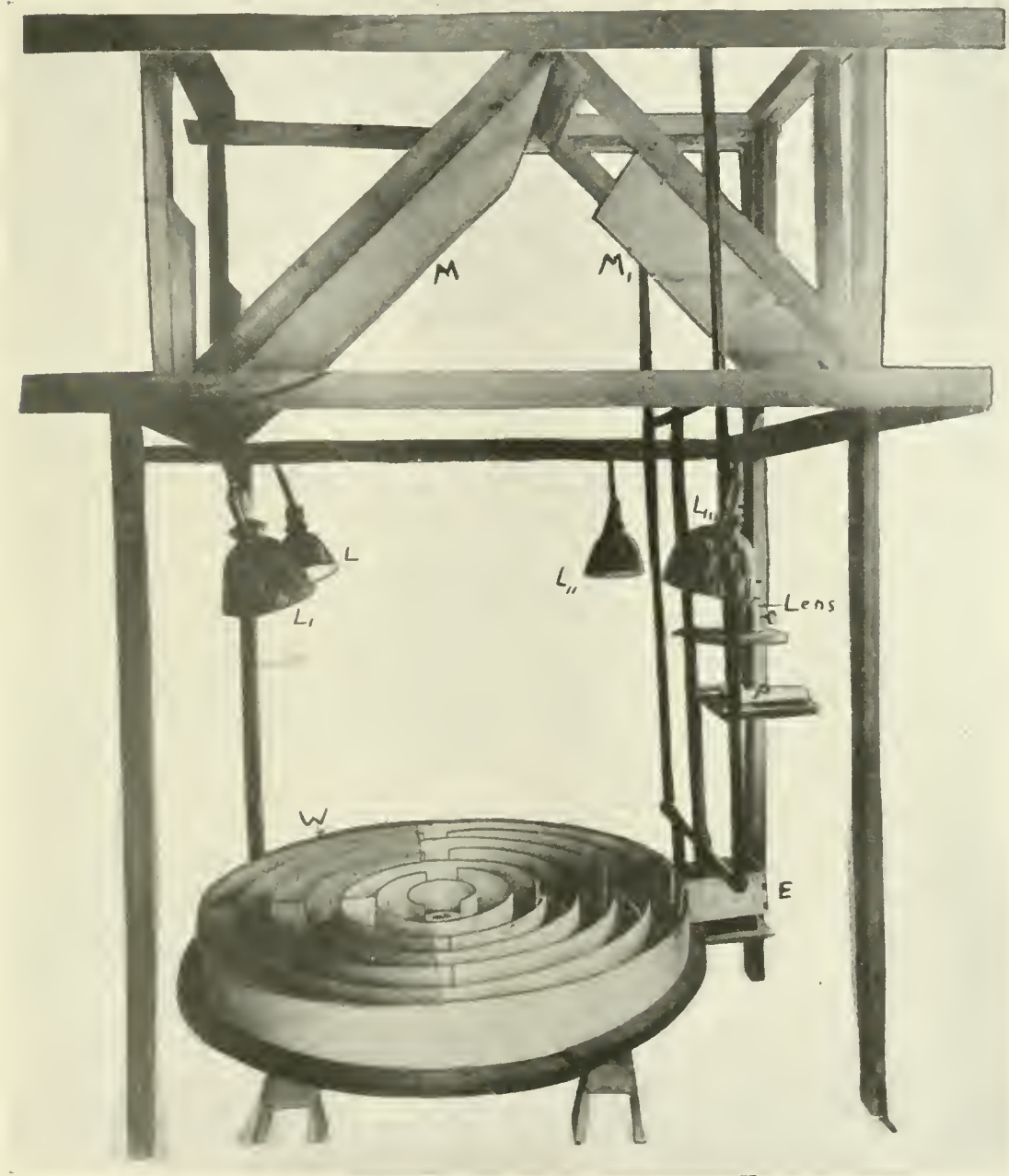


FIG. 2. CIRCULAR MAZE WITH CAMERA LUCIDA ATTACHMENT

Maze same as in figure 1. W , wire screen; M and M_1 , reflecting mirrors; L , L_1 , L_2 , and L_3 , electric lamps.

wide and 121 cm. in length, was placed at an angle of 45 degrees, directly over the center of the maze. At a certain distance from this mirror a second mirror M_1 , 61 cm. by 75 cm., is placed at an

angle of 45 degrees above the maze, at such a distance from the first mirror that the light reflected downward from it falls outside of the maze. Below M_1 , and in the light reflected from it, is placed a single acromat, 6 cm. in diameter and 50 cm. focus. The lens is placed in a barrel and the barrel is attached to a wooden disc 30 cm. in diameter. This board is attached to an iron collar which slides freely up and down and gives a very easy means of adjusting the size of the image. A path of circular paper is laid upon a wooden shelf, below the lens, and the distance is so adjusted that the reduced image of the maze is focused upon the paper. Extraneous light is excluded by means of a soft, dark flannel curtain, not shown in the figure. As may readily be seen from the figure, the maze must be illuminated quite highly in order to produce a clear image. This illumination is obtained by means of four powerful electric lamps, with opaque shades, placed symmetrically around the maze. By means of the camera lucida attachment, the movements of an animal in the maze can be traced upon white paper with a soft pencil. Such tracings are especially useful in the study of the learning of the maze problem, such as has been done by Miss Hubbert and others. In the present investigation, where the effect of drugs on the behavior of the animals was studied after the rats had been trained, the use of this attachment was not essential and it was therefore dispensed with in a great many of the experiments.

The study of the behavior of the rats in the circular maze is begun by placing an animal in the center of the maze and feeding it from the bowl *F* for three successive days. During these three preliminary feedings, which last from ten to fifteen minutes, the entrance γ is blocked off, so that the animal may not roam around. On the fourth day, the rat is placed in the cage *E*, then the trap-door *T* is raised and the animal allowed to enter the first alley. The animal then gradually learns to find its way to the center of the maze, when it is taken out and the experiment is repeated. Generally three trials are made on each day. For work with the maze, albino rats, which are very tame, must be employed. The animals must be handled gently with the hands, and under no circumstances must they be picked up with

forceps or similar instruments. The most suitable animals are found to be rats of approximately sixty to ninety days old. Older animals are apt to be sluggish, while very young rats do not learn the maze problem so readily. Ordinarily the albino rats learn the maze problem in about two weeks, and sometimes within a shorter period of time. An animal is considered to have solved the maze problem when it has learned to find its way into the center of the maze by the shortest route, that is, without any errors, on three successive trials. The technic of training is described more in detail by Hubbert (2).

ANALYSIS OF THE DATA FURNISHED BY THE MAZE

The maze problem enables the psychologist to study the mode of learning of a rat. In studying the effect of drugs, the maze problem can be utilized in two ways. Animals may be subjected to the influence of drug action first and then trained in the maze with the purpose of ascertaining the effect on the rate of learning. Again, animals may be first taught to solve the maze problem and then the effect of drugs is studied in reference to its influence on their behavior, memory-habit, etc. Furthermore, other data can be obtained from the maze, after administering drugs to rats, which may show the effect on neuromuscular coördination, and various somatic changes. As to exactly what the mechanism of learning the maze problem may be, the explanations given by various psychologists differ widely. Among the hypotheses which have been advanced to account for the reintegration of conduction paths in learning, there are at least three which stand out as rather opposed to one another in respect to the neural processes which they imply (4). The hypothesis suggested by Ladd and Woodworth (5) assumes inhibition of successive activities as the fundamental process which resulted in the selection and fixation of random activities. The second hypothesis, such as given by Angell (6), assumes nervous reinforcement as the fundamental process by which successive acts become linked together in habit-formation. The third hypothesis (Watson) depends chiefly upon the chance spreading of

nervous excitation, or the simultaneous activation of two afferent pathways, in such a way that the final common part of one is able to divert the discharge of the other and so bring about a permanent connection between itself and this afferent path (Max Meyer and Pawlow (7)). These hypotheses by no means exhaust the theoretical considerations of the maze problem (Dashiell) (8). For the study of drug action, however, the various theoretical considerations are of secondary importance and the data obtained are of a much more definite nature, as will be seen from the following exposition.

EXPERIMENTS WITH ANTIPYRETICS

Thirty-one rats were used altogether in the present investigation. The following drugs were administered: acetanilid, acetphenetidin, phenyl salicylate (salol), sodium salicylate, quinine sulphate, antipyrine and pyramidon. All the antipyretics were administered by injection of their solutions in physiological saline, either intraperitoneally or intramuscularly. Various doses of the different drugs were tried and observations were made on both the immediate and the late effects. It is hardly necessary to state that experiments were also made with injections of control solutions such as distilled water and plain physiological saline.

In each experiment the normal time of performance by the animal was first ascertained and if the animal was not in good condition it was not used. After establishing the normal running time for three successive trials the drug was injected, and half an hour later the animal was again allowed to run through the maze. Several hours later a second reading was made to determine the later effects of the drug and the general behavior of the animal was noted also on the following day.

EFFECT OF INDIVIDUAL DRUGS

Acetanilid. The experiments with this antipyretic are summarized in table 1. The table indicates the doses of the drug used; the number of trials, the time of performance and the

TABLE 1
Acetanilide

EXPERI- MENT NUMBER	DOSE <i>mgm.</i>	PERFORMANCE BEFORE DRUG			HALF HOUR AFTER DRUG			FOUR HOURS AFTER DRUG			EFFECT	LATE EFFECT
		Trials	Time	Errors	Trials	Time	Errors	Trials	Time	Errors		
I	1	3	29"	1E	3	29"		3	24"		Depression	Depressed 3 days
II	1	3	50"		3	68"	5E	3	52"			
III	1	3	16"		3	15"		3	15"			
IV	2	3	16"		3	39"	1E	3	21"		Depression	
V	2	3	24"		3	20"		3	18"			
VI	2	3	19"		3	15"		3	12"			
VII	3	3	172"		3	330"	1E	3	117"		Depression	Depressed next day
VIII	3	3	58"	2E	3	32"		3	29"		Depression	
IX	3	3	15"		3	37"		3	21"		Depression	
X	5	3	38"		3	137"		3	24"		Depression	
XI	5	3	60"		3	75"	2E	3	35"		Depression	
XII	5	3	33"		3	62"	3E	3	55"		Depression	Depressed next day
XIII	10	3	17"		3	52"	1E	3	15"		Depression	
XIV	10	3	33"		3	Stalled		3	Stalled		Depression	

number of errors committed before the administration of the drug; the number of trials, the time of performance and the number of errors half an hour after the injection of the drug; and another reading taken several hours later. The last two columns briefly express the effect of the drug on the same day and on the following days.

It will be noted that acetanilid produced in most cases a marked *depression* in the behavior of the rats as shown by their slower movements and the greater number of errors made in finding the way through the maze. In some cases a distinct depression was noted even on the following day or days.

Acetphenetidin or phenacetin. The effects of acetphenetidin or phenacetin are shown in table 2. The action of this drug was very much the same as that of acetanilid, though the depression was not as prolonged as after the first drug.

Salol and sodium salicylate. The influence of the salicylates is expressed in table 3. It was found that injections of sodium salicylate produced a slight retardation in running time. Phenyl salicylate or salol produced no effect and in some cases actually suggested a slight stimulation in the movements of the animals as may be seen in Table III.

Quinine. Injections of quinine sulphate were made and the effects of the drug are shown in table 4. It will be seen that quinine either produced no effect or caused a slight depression in the behavior and the neuro-muscular activity of the animals.

Antipyrine and pyramidon. Experiments with antipyrine and its derivative, dimethyl-amino-antipyrina, or pyramidon, are summarized in table 5. It is evident that antipyrine and pyramidon exerted the most depressant, or if we may so call it, narcotic effect on the behavior of the animals. Of these two, antipyrine seemed to be more depressant than pyramidon.

EFFECT OF MIXTURES

After experimenting with the individual drugs the following combinations were also studied: acetanilid plus acetphenetidin or phenacetin, sodium salicylate plus salol, phenacetin plus pyram-

TABLE 2
Phenacetine

EXPERI- MENT NUMBER	DOSE	PERFORMANCE BEFORE DRUG			HALF HOUR AFTER DRUG			THREE HOURS AFTER DRUG			EFFECT	LATE EFFECT
		Trials	Time	Errors	Trials	Time	Errors	Trials	Time	Errors		
I	<i>mgm.</i> 4	3	16"		3	16"		3	13"		Slight depression	
II	5	3	15"		3	20"		3	15"			
III	5	3	17"		3	19"		3	16"			
IV	5	3	16"		3	75"	2	3	15"		Depression	
V	5	3	16"		1	Stalled		3	19"		Depression	
VI	5	3	18"		1	23"	1	3	24"	3	Depression	

TABLE 4
Quinine

EXPERI- MENT NUMBER	DOSE	PERFORMANCE BEFORE DRUG			HALF HOUR AFTER DRUG			THREE HOURS AFTER DRUG			EFFECT	LATE EFFECT
		Trials	Time	Errors	Trials	Time	Errors	Trials	Time	Errors		
I	<i>mgm.</i> 2.5	3	15"		3	15"		3	14"		Depressed Depressed Depressed	
II	5	3	16"		3	19"		3	15"			
III	5	3	17"		3	18"		3	18"			
IV	5	3	27"		3	62"	1	3	43"			
V	5	3	28"		3	44"	1	3	24"			
VI	5	3	42"		1	Stalled		3	30"			

TABLE 3
Salol and sodium salicylate

EXPERI- MENT NUMBER	DRUG	DOSE <i>mgm.</i>	PERFORMANCE BEFORE DRUG			HALF HOUR AFTER DRUG			THREE HOURS AFTER DRUG			EFFECT	LATE EFFECT
			Trials	Time	Errors	Trials	Time	Errors	Trials	Time	Errors		
I	Salol	1	3	18"		3	13"		3	14"		Stimulation	
II	Salol	1	3	19"		3	14"		3	13"			
III	Salol	1	3	19"		3	19"		3	19"			
IV	Salol	1	3	42"		3	26"		3	23"			
V	Salol	1	3	21"		3	31"		3	28"			
VI	Salol	1	3	24"		3	17"	1	3	15"		Slight depression	
VII	Salol	5	3	17"		3	17"		3	19"			
VIII	Salol	5	3	29"		3	35"	1	3	22"			
IX	Salol	5	3	18"		3	21"		3	23"			
X	Salol	5	3	18"		3	14"		3	12"			
XI	Salol	8	3	14"		3	16"		3	13"		Slight depression Slight depression Depression Depression	
XII	Salol	8	3	46"		3	48"		3	39"			
XIII	Na salicylate	5	3	19"		3	18"		3	17"			
XIV	Na salicylate	5	3	15"		3	22"	2	3	14"			
XV	Na salicylate	5	3	22"		3	29"		3	21"			
XVI	Na salicylate	10	3	32"		3	54"		3	29"		Depression	
XVII	Na salicylate	10	3	25"		3	47"	2	3	36"			

TABLE 5
Antipyrine and pyramidon

EXPERI- MENT NUM- BER	DRUG	DOSE	PERFORMANCE BEFORE DRUG			HALF HOUR AFTER DRUG			THREE HOURS AFTER DRUG			EFFECT	LATE EFFECT
			Trials	Time	Errors	Trials	Time	Errors	Trials	Time	Errors		
I	Antipyrine	2	3	37"		3	70"		3	50"	1	Depressed	Depressed next day
II	Antipyrine	5	3	16"		3	47"		3	15"		Depressed	
III	Antipyrine	5	3	52"	1	3	270"	6	3	216"	3	Depressed	
IV	Antipyrine	5	3	25"		2*	74"	2	3	28"		Depressed	Depressed next day
V	Antipyrine	5	3	14"		3	31"	3	3	13"		Depressed	
VI	Antipyrine	10	3	56"		1	Stalled		3	113"	3	Depressed	
VII	Pyramidon	5	3	15"		3	34"	2	3	16"		Depressed	
VIII	Pyramidon	5	3	14"		3	15"		3	15"			
IX	Pyramidon	5	3	16"		1	Failed		3	17"		Depressed	
X	Pyramidon	5	3	22"		1	Failed		3	37"		Depressed	
XI	Pyramidon	10	3	28"		3	280"	5	3	34"		Depressed	
XII	Pyramidon	10	3	47"	1	3	41"		3	38"		Depressed	

* Refused to move on third trial.

VI	Na salicylate + Salol	2.5 2.5	3	19"	3	16"	3	15"	Very slight	None
VII	Na salicylate + Salol	2.5 2.5	3	19"	3	20"	3	17"		
VIII	Na salicylate + Salol	5.0 5.0	3	27"	3	29"	3	24"		
IX	Na salicylate + Salol	5.0 5.0	3	30"	3	31"	3	33"		
X	Na salicylate + Salol	5.0 5.0	3	51"	3	49"	3	37"		

[illegible]

TABLE 8
Acetanilide and sodium bicarbonate

EXPERI- MENT NUM- BER	DRUG	DOSE	PERFORMANCE BEFORE DRUG			HALF HOUR AFTER DRUG			THREE HOURS AFTER DRUG			EFFECT	LATE EFFECT
			Trials	Time	Errors	Trials	Time	Errors	Trials	Time	Errors		
I	NaHCO ₃	6	3	16"		3	17"		3	15"		None	None
II	NaHCO ₃	6	3	118"		3	113"		3	97"		None	
III	NaHCO ₃	6	3	33"		3	21"		3	23"		None	
IV	NaHCO ₃	6	3	21"		3	27"		3	28"		None	
V	NaHCO ₃	6	3	82"	4	3	18"		3	17"		None	
VI	NaHCO ₃	6	3	221"		3	197"		3	147"		None	
VII	Acetanilide 4 mgm. NaHCO ₃ 6 mgm.	10	3	18"		3	15"		3	16"		None	Slight stimulation
VIII	Acetanilide 4 mgm. NaHCO ₃ 6 mgm.	10	3	35"		3	19"		3	16"			
IX	Acetanilide 5 mgm. NaHCO ₃ 6 mgm.	11	3	30"	1	3	25"		3	24"		None	Stimulation*
X	Acetanilide 5 mgm. NaHCO ₃ 6 mgm.	11	3	125"		3	67"		3	41"			
XI	Acetanilide 5 mgm. NaHCO ₃ 6 mgm.	11	3	222"		1	Stalled		1	856"		Depressed†	None
XII	Acetanilide 10 mgm. NaHCO ₃ 6 mgm.	16	3	15"		3	15"		3	15"			

* Probably hunger stimulation.
† Rat was ill when experimented with. Practically normal the following day.

idon, acetanilid plus pyramidon, acetanilid plus salol, and acetanilid plus sodium bicarbonate.

The effects of combining equal parts of acetanilid and phenacetin, and sodium salicylate and salol are shown in table 6. A comparison of the results obtained with the results obtained after administering the individual drugs, shows that these combinations exert a somewhat less depressant effect than double doses of the individual components when given alone.

Combinations of phenacetin and pyramidon and acetanilid and pyramidon also show a distinctly less depressant effect than the individual components do when given alone (see table 7).

The combination of acetanilid and salol also shows a slightly synergistic effect (see table 7).

It was of special interest to inquire into the effect of the combination of acetanilid with sodium bicarbonate, in as much as such combinations have been found by Hale (9) and by Macht, Greenberg and Isaacs (10) to be less toxic than acetanilid alone. Table 8 shows that small doses of sodium bicarbonate produce no change in the psychological response of the rats. In the same table it will be seen that the combination of acetanilid and sodium bicarbonate produces a distinctly less depressant action than acetanilid alone (see table 1).

DISCUSSION

An examination of all the results obtained leads to the conclusion that the various antipyretics studied all produce either no effect or a depression in the psychological response of the albino rats as indicated by their behavior in the maze. Injections of salol gave results which might be regarded as a slight stimulation, but that is doubtful. Of the various groups of antipyretics studied, antipyrine and pyramidon were the most depressant or (using the term in its broadest sense) narcotic. Next come acetanilid and phenacetin. The salicylates were but slightly depressant, if at all, and quinine was also mildly depressant after large doses.

It may be well to call attention to the fact in this place, that those animals which were sluggish under normal conditions showed the greatest depression after injections of the drugs

It is interesting to note that some of the combinations studied exhibited a distinct synergistic effect, this was specially marked in the case of acetanilid and salol and in the case of acetanilid plus sodium bicarbonate. These combinations produced much less depression than the individual components when given alone.

It is interesting, furthermore to compare the effect of the antipyretics with those of the opium alkaloids on the behavior of rats in the maze. The results obtained in the two investigations are not very different. In both researches the effects of the combinations were less depressant than that of the individual drugs. It must be noted, however, that in the case of morphine a primary stimulation was observed in a few cases, while no such primary stimulation (with the possible exception of salol) occurred after injections of antipyretics.

Lastly, the authors wish to state that even after very large and repeated injections of the antipyretics the animals although greatly depressed, eventually completely recovered from the effects of the poisons.

CONCLUSIONS

1. The effects of various antipyretics individually and in combination with each other were studied on the behavior of rats in the circular maze.

2. It was found that the various antipyretics depressed the behavior and the memory habit of the rats; antipyrine and pyramidon being the most powerful in this respect.

3. Combinations of various antipyretics produced less depression than the individual components when given alone.

4. In all cases even after very large doses of the drugs, the animals eventually regained their normal activity.

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OPTICAL ISOMERS

VII. HYOSCINES AND HYOSCYAMINES

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In 1905, Dr. Roy Peebles and I (1) compared the effects of two isomeric hyoscines, the ordinary laevorotary form and an optically inactive hyoscine derived from it by heating with alkali by Gadamers method (2). The latter was assumed to be the racemic form of the original *l*-hyoscine, and by comparing their relative activity we deduced that of the *d*-isomer. Since that time considerable progress has been made in the chemistry of hyoscine. Tutin (3) was able to resolve benzoyl-oscine into its *l* and *d* components, thus showing that, unlike tropine, oscine is not internally compensated, which is in agreement with the results of Schmidt and Hess on the structural formula of oscine. Both components of hyoscine, the tropic acid and the oscine, thus contain asymmetrical carbon atoms, and King (4) has pointed out that it is therefore theoretically possible that hyoscine may exist in ten or even eleven different isomeric forms. Of these, four are simple optically active bases:

- | | |
|---------------------------------------|--|
| (1) <i>l</i> -troyl- <i>l</i> -oscine | (3) <i>d</i> -troyl- <i>l</i> -oscine |
| (2) <i>l</i> -troyl- <i>d</i> -oscine | (4) <i>d</i> -troyl- <i>d</i> -oscine; |

four are partially racemised:

- | | |
|--|---|
| (5) <i>l</i> -troyl- <i>dl</i> -oscine | (7) <i>dl</i> -troyl- <i>l</i> -oscine |
| (6) <i>d</i> -troyl- <i>dl</i> -oscine | (8) <i>dl</i> -troyl- <i>d</i> -oscine; |

two are racemates

(9) (*l*-troyl *l*-oscine) (*d*-troyl *d*-oscine) = *dl*-troyl-*dl*-oscine A.
(10) (*l*-troyl *d*-oscine) (*d*-troyl *l*-oscine) = *dl*-troyl-*dl*-oscine B.
and one (11) is a double racemate containing both the *dl*-troyl-oscines A and B.

On crystallisation of the inactive or partially active hyoscyne with *d*-αbromo-πcamphorsulphonic acid, King succeeded in isolating a *d*-hyoscyne identical with the ordinary *l*-hyoscyne except in the direction of optical rotation. A mixture of equal parts of these *l*- and *d*-hyoscines gave an optically inactive form identical with the atropine base of Hesse. The *l*-hyoscyne on hydrolysis with acid or alkali yields *l*-tropic acid and *dl*-oscine, while benzoyl-*d*-oscine gives *d*-oscine under the same conditions, and *d*- and *l*-oscine are not racemised by acids or alkalies. King draws the conclusion that ordinary *l*-hyoscyne is partially racemised in nature, and assigns it the form *l*-tropyl-*dl*-oscine, the corresponding *d*-hyoscyne which he isolated being *d*-tropyl-*dl*-oscine.¹ The *dl*-hyoscyne or optically inactive hyoscyne obtained from *l*-hyoscyne by alkalies is therefore a mixture of these, the double racemate (no. 11) containing all four of the simple optically active bases.

In short, the *l*-hyoscyne in ordinary use as isolated from the plants consists of equal parts of *l*-tropyl-*d*-oscine and *l*-tropyl-*l*-oscine. The enantiomorphous *d*-hyoscyne obtained by King consists of equal parts of *d*-tropyl *d*-oscine and *d*-tropyl-*l*-oscine, while the *dl*-hyoscyne or atropine, formed by the action of alkalies on *l*-hyoscyne, is made up of equal parts of each of the four primary hyoscines—*l*-tropyl-*l*-oscine, *l*-tropyl-*d*-oscine, *d*-tropyl-*l*-oscine and *d*-tropyl-*d*-oscine. None of these four primary hyoscines has been examined chemically or pharmacologically and none of them is at present available.

I have been able to examine the *l*- and *d*-hyoscyne of Dr. King through the kindness of Professor F. Lee Pyman and Dr. T. A. Henry, former and present directors respectively of the Wellcome Chemical Research Laboratories, and desire to express my indebtedness to them. In my previous experiments on hyoscyne, I could only infer the action of the dextro-rotary base from a comparison of the effects of the enantiomorphous *l*-hyoscyne with that of the racemic *dl*-form. I am now able to contrast the two active isomers directly. These two isomers are identical in character except

¹ I shall employ the terms '*l*-hyoscyne' and '*d*-hyoscyne' to indicate *l*-tropyl-*dl*-oscine and *d*-tropyl-*dl*-oscine respectively in this paper; *dl*-hyoscyne will indicate the double racemate.

that the *l*-hyoscine has $[\alpha]_D = -25.9$, while the *d*-hyoscine has $[\alpha]_D = +26.3$ (King).

Peebles and I found that the *l*-hyoscine affected the peripheral ends of the chorda tympani in the salivary glands and of the vagus in the heart about twice as powerfully as the *dl*-variety and drew the conclusion that while the *l*-form is intensely active here, the *d*-form is almost or quite inactive. The action of the *l*- and *dl*-bases on the motor nerve ends in the frog and on the central nervous system in man and other mammals was identical, so that we concluded that the *l*- and *d*-hyoscine were equivalent in regard to these organs. Hug (5) comparing the same two forms, *l*-hyoscine and Gadamer's *dl*-hyoscine, found that the former acts about twice as strongly on the iris as the latter, thus confirming our results. His statement that *l*-hyoscine is three to four times as powerful as the *dl* form in paralysing the rabbit's vagus is obviously due to an imperfect method of investigation, for, as he admits himself, one half of the *dl*-hyoscine is *l*-hyoscine, and the racemic form must therefore be at least half as active in the tissues as the laevorotary (6). In a number of experiments made on the rabbits vagus after the method of Hug, I found it quite unreliable in estimating the quantity of hyoscine injected. Cloetta's note (7) in support of Hug's statement is not illuminating; racemic hyoscine is a simple mixture of the two active forms in equal parts and therefore possesses the action of each of these independently; unless the two active forms are antagonists pharmacologically, the racemic form must possess at least half the action of the laevorotary isomer.

Smith (8) comparing *l*-hyoscine with *dl*-hyoscine found the former considerably less toxic than the latter to frogs, while they were equally poisonous to mice. In combination with morphine, the laevorotary base proved more toxic to mice than *dl*-hyoscine. It may be remarked that the *l*-scopolamine hydrobromide used by Smith gave an optical rotation of only -19.5° , while that given for the pure hydrobromide by King is -25.9 ; the specimen examined by Smith is therefore of only 75 per cent purity.

The only other comparison of the action of the hyoscines with which I am acquainted is that of Macht (9) on the effects of the

isomers on the movements of the pig's ureter; he states that *l*-hyoscine stimulates the movements, while *d*-hyoscine inhibits them, and the *dl*-form also inhibits. The same difference was observed in the effects of the hyoscyamines, the *l*-stimulating, the *d*-inhibiting, while the *dl*-form (atropine) first stimulates and then inhibits. But Liljestrand (10) points out that the reaction of the surviving gut to atropine varies extraordinarily in character, some preparations responding with increased activity, others with decreased, and no explanation of this variation has been suggested. He was unable to detect any qualitative or quantitative difference in the action of atropine and *l*-hyoscyamine on the movements of the surviving gut of the rabbit. The effects of atropine on the gut are obviously of the same nature as those on the ureter, and Liljestrand's results are so different from those of Macht that further observations seem desirable, in regard to both the hyoscyamines and the hyoscines.

I first compared the action of the two hyoscines on the salivary glands of the dog, the same animal being used as was employed for the investigation of the tropeines (11); its weight was 6 kgm. A permanent salivary fistula was formed and the saliva secreted in successive periods of five minutes was collected on weighed pledgets of cotton wool and its amount ascertained by reweighing. The hyoscine was injected subcutaneously and ten minutes later 5 mgm. of pilocarpine hydrochloride was similarly injected. The course of the secretion was followed for forty minutes and the graph of the secretion was compared with those drawn from similar experiments performed with varying amounts of the enantiomorphous base at intervals of two days or more.

In the first series (fig. 1) the drug was injected as hydrobromide but the quantities are calculated as base. Each graph represents the average of two or three experiments in which the same quantities were injected. In this series, the graph of 0.5 mgm. *d*-hyoscine lies between that of 0.02 and 0.03 mgm. *l*-hyoscine but nearer the latter. It may be taken that 0.5 mgm. *d*-hyoscine offers the same antagonism to pilocarpine as 0.027 mgm. *l*-hyoscine, that is the laevorotary is about eighteen times as active as the dextrorotary isomer.

Another estimation was later made on another dog with permanent salivary fistula and weighing 15.5 kgm. Here (fig. 2) 1 mgm. *d*-hyoscine hydrobromide proved rather less efficient than 0.07 mgm. *l*-hyoscine hydrobromide, much more efficient than

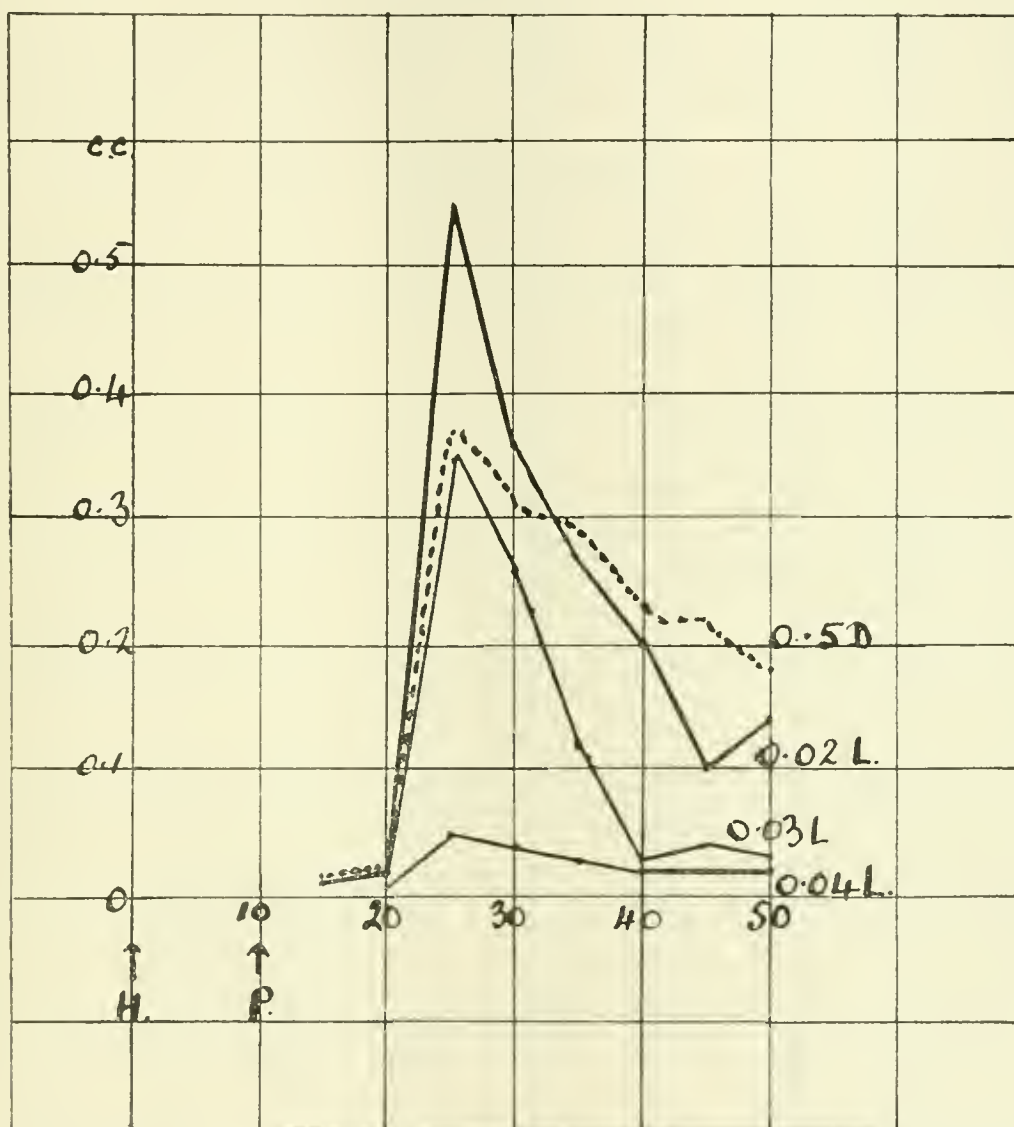


FIG. 1. GRAPHS OF THE SECRETION OF SALIVA BY A DOG FROM 5 MGm. OF PILOCARPINE NITRATE INJECTED SUBCUTANEOUSLY AT *P*, PRECEDED BY HYOSCINE HYDROBROMIDE AT *H*

The unbroken lines represent the secretion from pilocarpine after 0.02, 0.03, and 0.04 mgm. *l*-hyoscine; the dotted line that from pilocarpine after 0.5 mgm. *d*-hyoscine. The *d*-hyoscine hinders the pilocarpine secretion more than 0.02 *l*-hyoscine, but less than 0.03 *l*- (quantities given in terms of the base). The time is given in minutes along the abscissa, the amounts of saliva in cc. along the ordinate.

0.035 mgm. and much less efficient than 0.1 mgm. It may be taken as equal to 0.06 mgm. *l*; that is, the relative power of *d*- and *l*-hyoscine in antagonising pilocarpine in the salivary glands is about 1:16.

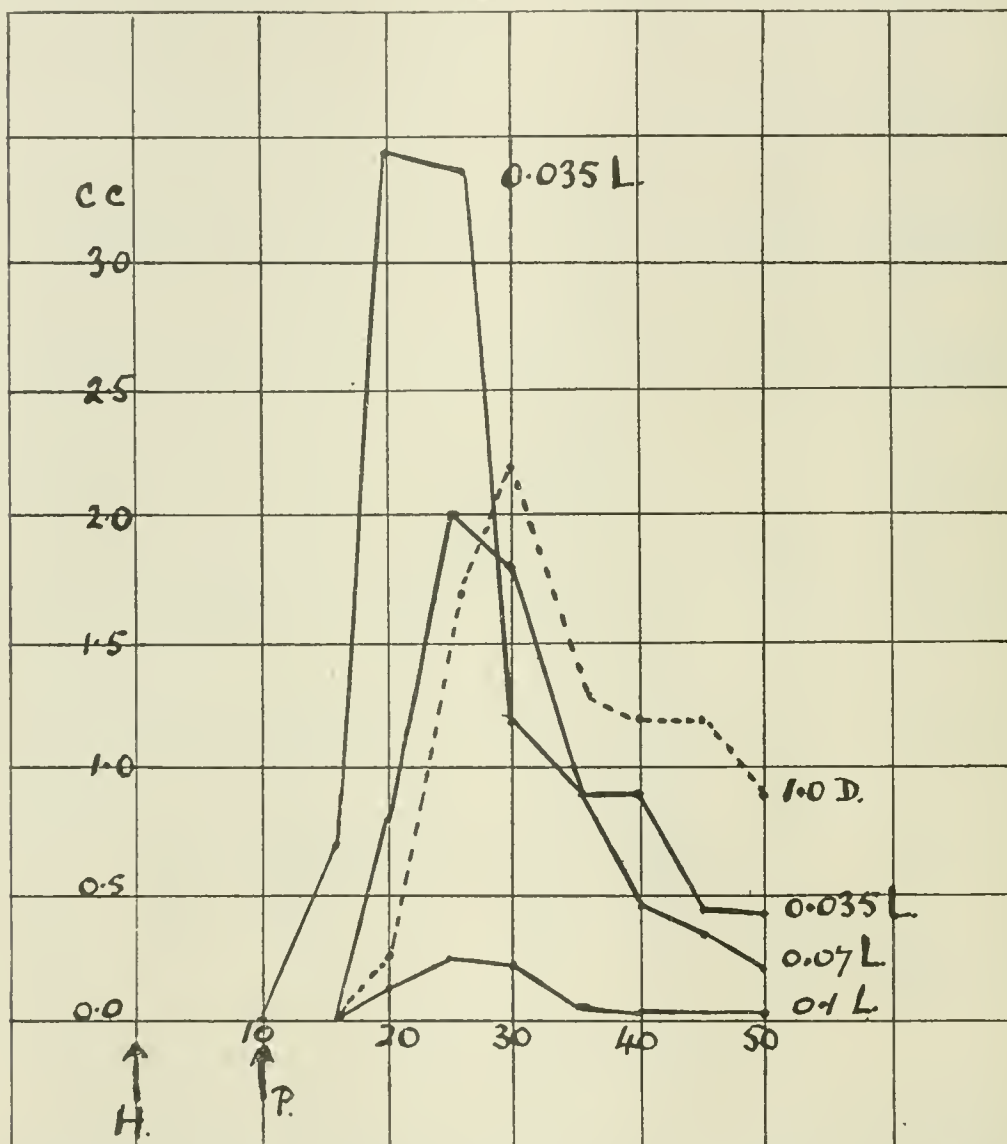


FIG. 2. GRAPHS OF THE SECRETION OF SALIVA BY ANOTHER DOG FROM 5 MGM. PILOCARPINE NITRATE INJECTED SUBCUTANEOUSLY AT P, PRECEDED BY HYOSCINE HYDROBROMIDE AT H

The unbroken lines represent the secretion from pilocarpine after 0.035, 0.07 and 0.1 mgm. *l*-hyoscine, the dotted line that after 1 mgm. *d*-hyoscine. The *d*-hyoscine graph is the average of four experiments, the 0.07 *l*-that of two experiments, while those of 0.035 and 0.1 mgm. *l*-were each derived from a single experiment. In this experiment the quantities are given in terms of the hydrobromide and are not calculated as base.

The two sets of experiments are in close accord and indicate that *l*-hyoscine is about sixteen to eighteen times as powerful as *d*-hyoscine. In our previous paper on hyoscines, *dl*-hyoscine was stated to be approximately half as strong as *l*-hyoscine and from this it was deduced that *d*-hyoscine was practically inactive. The very slight action of the *d*-isomer could not be detected when it was injected along with the *l*-base, the difference (6 per cent) lying within the limits of error of the method.

The pulse was not accelerated in any of these observations either by *l*-hyoscine (0.1 mgm.) or by *d*-hyoscine (1 mgm.). The quantity injected was always sufficient to prevent the nausea and vomiting which were present when pilocarpine was injected alone.

Macht's results with the isomers on smooth muscle seemed to demand further investigation, and I have therefore tested the action of *d*- and *l*-hyoscine on pieces of rabbit's intestine suspended in oxygenated Ringer's solution or Tyrode's solution. The method of recording was that generally used in pharmacological laboratories and requires no special comment; the glass tube containing the bowel held 50 cc. of Ringer's solution. Two solutions, one containing 1 mgm. of the hydrobromide of *l*-hyoscine in the cubic centimeter, the other an equal amount of the *d*-salt were contrasted by dropping them alternately into the Ringer solution. After each observation the tube was emptied, refilled with Ringer solution, again emptied after a minute and refilled for the application of an equal amount of the isomer of opposite sign. In some experiments 1 mgm., in others 0.5 mgm. of hyoscine was used for each administration.

In my experiments the effects of the hyoscines varied greatly. The first application of either to the freshly suspended gut sometimes caused marked stimulation, the size of the contractions increasing very greatly and the tonus rising to some extent. The second application (after washing) caused less change and each subsequent application still less, until finally very little increase could be made out from either (experiment I, fig. 3). In figure 3 the first three tracings from experiment I, are given; the increase in movement is less marked, while the latent period increases with each application.

Experiment 1. Rabbit's gut suspended in 50 cc. Ringer's solution, oxygen bubbled through throughout. Temperature 38°. Tube emptied and thoroughly washed between each observation.

OBSERVATION	INJECTION		HEIGHT OF CONTRACTION	
			Normal	Under hyoscine
		<i>mgm.</i>	<i>mm.</i>	
1	D.	1	7	38
2	L.	1	7	20
3	D.	1	7	12
4	L.	1	6	11
5	D.	1	7	11
6	L.	1	4	7
7	L.	1	3	5

In other experiments neither base caused any appreciable increase in the contraction, each application of either being followed by some weakening and slowing of the contraction. And the two isomers had the same effect when averages of the results of several applications were compared. In no case was I able to confirm Macht's statement that the one stimulated while the other depressed the bowel movement, both having the same action either in reducing or increasing the movement according to the condition of the gut.

In one experiment the bowel was suspended in oxygenated Tyrode's solution which was also used to dissolve the hyoscine hydrobromide, and the latter was carefully warmed to the temperature of the bath. In this case, I found the bowel movements extraordinarily little affected by the alkaloids. Thus 20 mgm. of the hydrobromide of either alkaloid added to the 50 cc. in which the bowel was suspended had no appreciable effect. Even 40 mgm. caused only a transient depression, which soon disappeared even when the alkaloids were still present in the fluid (fig. 4).

The action on the bowel muscle therefore seems to vary greatly in different animals, but both alkaloids have the same effect, whatever the nature of the response may be; the two hyoscines thus resemble each other in this respect; Liljestrand found atropine and *l*-hyoscyamine similar in action.

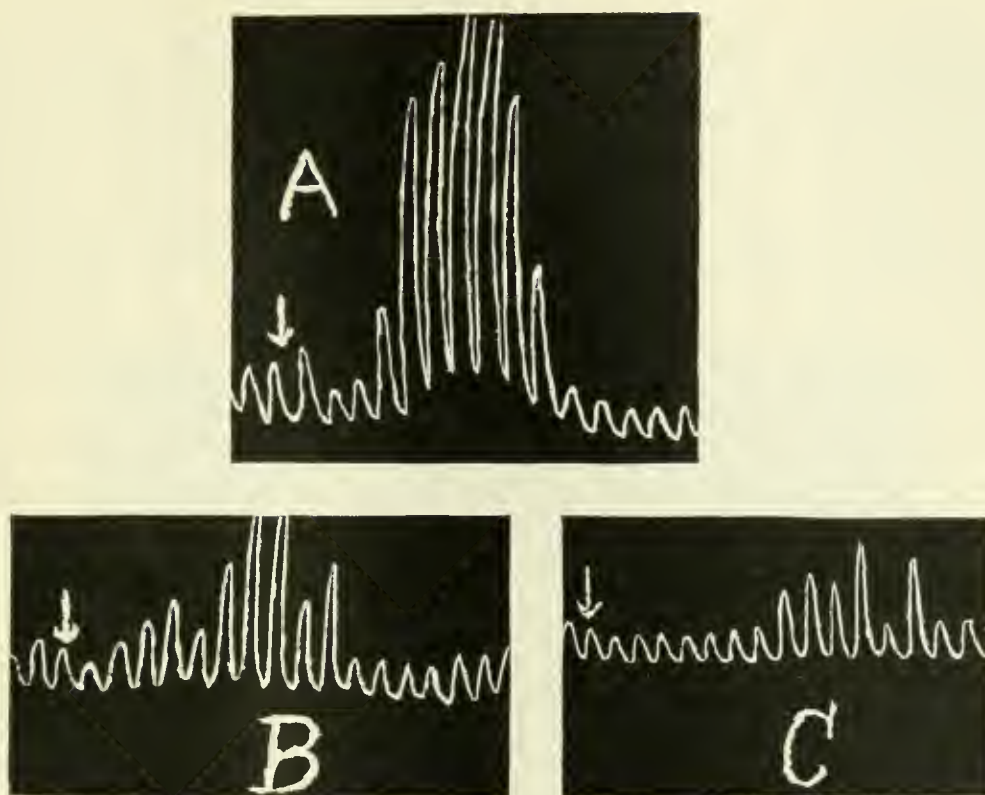


FIG. 3. EFFECT OF HYOSCINES ON THE MOVEMENTS OF THE SURVIVING BOWEL IN 50 CC. OF OXYGENATED RINGER'S SOLUTION (EXP. 1)

The arrows indicate the application of the hyoscines; A, 1 mgm. *d*-hyoscine; B, 1 mgm. *l*-hyoscine; C, 1 mgm. *d*-hyoscine.

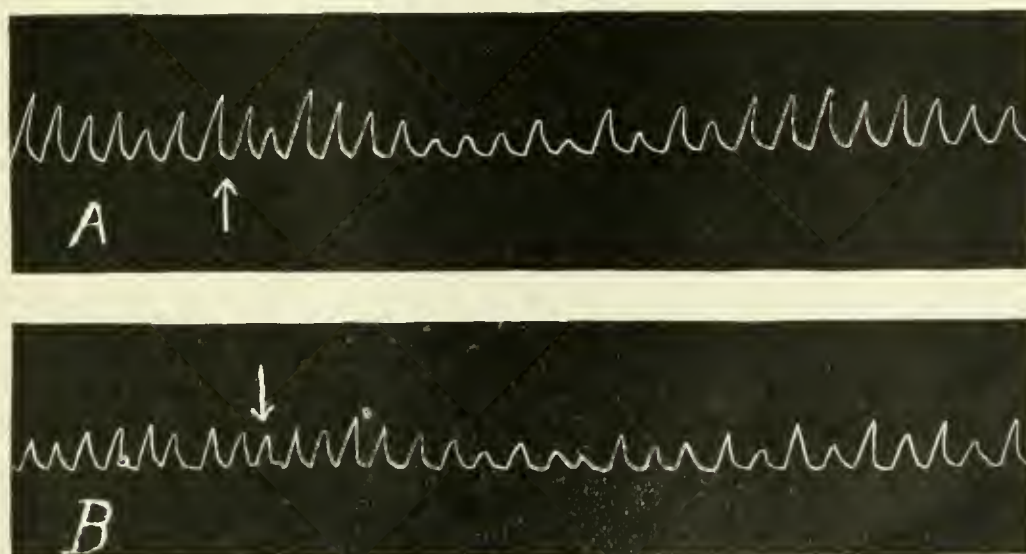


FIG. 4. TRACING OF SURVIVING INTESTINE OF RABBIT IN 50 CC. OF TYRODE'S SOLUTION

In A 40 mgm. of *l*-hyoscine hydrobromide was added to the solution at the arrow. In B 40 mgm. of *d*-hyoscine hydrobromide was added.

Another series of observations was made on the relative power of the hyoscines in antagonising the action of pilocarpine on the bowel. I am not aware of any previous experiments having been done with the hyoscines, but Liljestrand compared *l*-hyoscyamine with atropine as antagonists to pilocarpine and found comparatively little difference. He concludes that *l*-hyoscyamine may be more effective than atropine, it is certainly less than twice as powerful. He seems to have done only a limited (*beschränkten*) number of experiments and his results may be due in part to his having used alkaloids which were admittedly not very pure. But it may be questioned whether his method admitted of very close approximations. He first induced the characteristic movements with pilocarpine and then relieved them by dropping in varying amounts of atropine or hyoscyamine and measuring the extent of relaxation occurring within a definite time. In the tracings given on p. 122 of his communication it is difficult to estimate the success of the antidote; thus the difference between the effects of 0.0004 mgm. and 0.0002 mgm. atropine seems no greater than that between the effects of two administrations of 0.0003 mgm. atropine.

In my experiments, pieces of rabbit's gut were suspended in 250 cc. of Ringer's solution at 37°C., oxygen bubbling through. One-half cubic centimeter of a solution of pilocarpine nitrate containing 1 mgm. to 1 cc. was found to cause a marked increase in tonus with increased strength of contraction. The Ringer was then removed and the preparation washed with fresh fluid. A small dose of *l*-hyoscine was now added, and after two minutes the standard dose of 0.5 mgm. pilocarpine. After the movements had become fairly regular, the fluid was removed, the preparation washed and the same estimation done for *d*-hyoscine and pilocarpine. A series of such estimations was made alternately with the two isomers, the quantities of each being varied until the resultant curves approximated each other in character sufficiently closely. The final results of one experiment are given in figure 5. (The solution of *l*-hyoscine was made up to contain 0.001 mgm. of the hydrobromide, that of the *d*-hyoscine to contain 0.01 mgm.)

From this experiment it is clear that 0.03 mgm. of *d*-hyoscine hydrobromide is nearly equivalent to 0.002 mgm. *l*-hyoscine hydrobromide in antagonising 0.5 mgm. pilocarpine nitrate, and is much weaker than 0.003 mgm. of the *l*-base. In other experiments a similar ratio between the hyoscines was found. The *l*-hyoscine is thus about fifteen times as powerful as the *d*- in

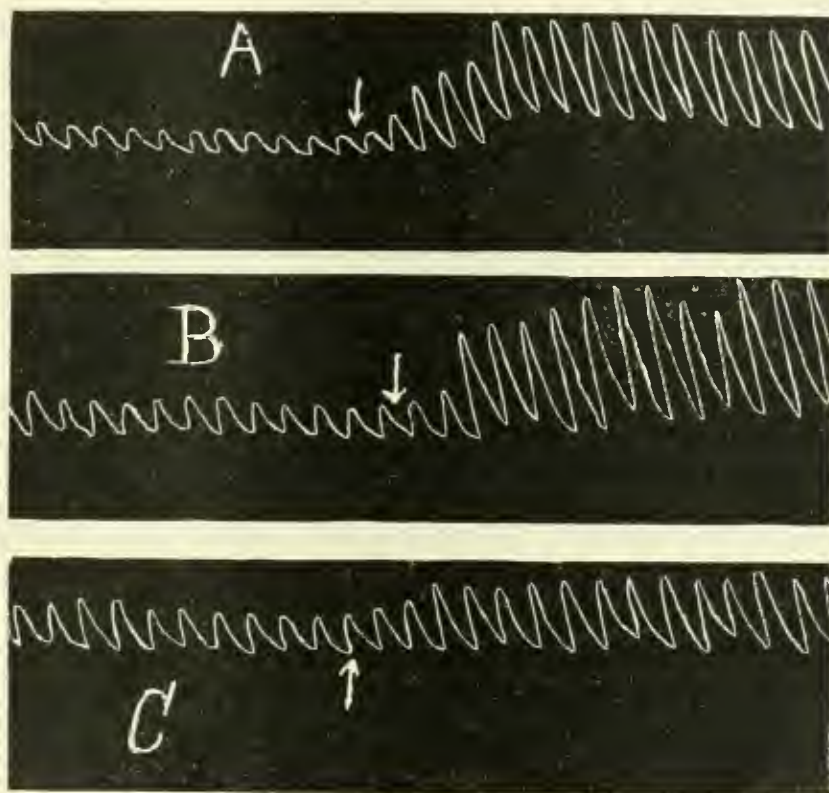


FIG. 5. RECORDS OF MOVEMENTS OF A PIECE OF SURVIVING INTESTINE OF RABBIT IN RINGER'S SOLUTION

In each tracing 0.5 mgm. pilocarpine nitrate was added where indicated by the arrow. In *A* this was preceded (immediately before the curve began) by 0.002 mgm. *l*-hyoscine hydrobromide, in *B* by 0.03 mgm. *d*-hyoscine hydrobromide, and in *C* by 0.003 mgm. *l*-hyoscine hydrobromide.

opposing pilocarpine in the gut; i.e., the ratio is of the same order as in the salivary gland. On the other hand, they appear to be equally powerful in causing the stimulation or depression of the intestinal movements described by Magnus. The inference may be drawn that the hyoscines act on receptors of different characters in inducing these two changes; those involved in the pilocarpine

action and in its antagonism in the bowel are of the same nature as those in the salivary glands; they differentiate between isomers of different sign and are therefore themselves optically active. Those receptors which are involved in the stimulation and depression effects in the bowel fail to make this distinction and may therefore be not optically active.²

The general action of the alkaloids was compared in frogs (*Rana temporaria*) of about 20 grams in weight, which were chosen in pairs not differing more than 2 grams in weight, one of each pair receiving the *l* and the other an equal amount of the *d*-hyoscine hydrobromide; the drug was injected into the anterior lymph sac, through the floor of the mouth. The lowest effective dose of each was about 10 mgm. which caused the same partial curara action as was observed by Peebles and myself, and which it is unnecessary to describe again. No difference was observed between the isomers in this dose. With larger doses (20 mgm. = 1 mgm. per gram) the peripheral paralysis was greater but still incomplete. Here again the symptoms induced by the isomers were identical in kind and degree in the earlier part of the experiment but the recovery from the *l*-hyoscine was considerably quicker than that from the *d*-. When 40 mgm. were injected (2 mgm. per gram) complete curara paralysis occurred, no movement being elicited by stimulation of the nerve plexus, while the muscles responded with normal tetanus to direct stimulation. The heart was slow and the circulation in the web slow and imperfect, but frogs recovered from these large doses completely in the course of twenty-four to forty-eight hours. The *d*-hyoscine acted for a longer period than the *l*, some weakness and clumsiness in movement being detected in the frog under it for as long as twenty-four hours after its opposite number under *l*-hyoscine had completely recovered. In other respects the effects from these large doses were identical. In no case was there any increase in the reflex excitability such as occurs in the late stages of poisoning

² In one experiment the two isomeric hyoscyamines were contrasted in the same way as the hyoscines. The *l*-hyoscyamine was found to be more than ten times as powerful as the *d*-hyoscyamine in antagonising pilocarpine; no nearer approximation was possible as the experiment had to be broken off.

with atropine or *d*-hyoscyamine (12). The stimulant action on the frog's spinal cord is confined to the tropine derivatives and does not extend to the oscine series. None of the frogs showed any evidence of depression of the central nervous system, although the milder degrees of narcosis are difficult to detect in the presence of peripheral paralysis. Still, reflex movements were obtained as easily as in normal animals, as long as the curara action was not complete. The hyoscine action on the temporaria is therefore identical with that on the American frogs described in our previous paper. The hyoscines also differ from the hyoscyamines in the shortness of their action, the symptoms generally beginning to pass off in six to eight hours after the injection and the animals resuming their normal movements completely within twenty-four hours except from very large doses, such as 2 mgm. per gram. This shortness of action is especially marked under *l*-hyoscine, for even after 2 mgm. per gram, which caused complete curara paralysis in an hour, the animal was quite normal after twenty-three hours. The corresponding animal under *d*-hyoscine was paralysed as completely at the end of an hour, and had partially recovered in twenty-three hours, but the movements were still weak and clumsy after thirty hours and the normal condition was only attained next morning; that is after forty-eight hours. The *d*-hyoscine thus differs from the *l*-isomer in possessing a more prolonged curara action on the frog, which however is identical in character. This is apparently due to its being destroyed or eliminated more slowly. An attempt was made to estimate roughly the rate of excretion in the frog, by collecting the fluid from the dish, evaporating it and extracting the residue with alcohol. The remainder on evaporating off the alcohol was too small to admit of further analysis and was practically the same whether *d*- or *l*-hyoscine had been injected. As far as it goes, this suggests that the difference in the duration of action arises from a difference in the rate of destruction rather than from one in the rate of excretion. Optical isomers have been shown to differ in the rate of oxidation in the tissues in several cases, and the hyoscines afford only a further example of this behaviour. The more prolonged action of the *d*-base may perhaps explain

in part the observation of Smith (8) that *dl*-hyoscine is more toxic to frogs than the *l*-form.

This difference in the duration of action of the two hyoscines in the frog shows some analogy with the effects seen under the hyoscyamines. Here also the action of atropine (*dl*-hyoscyamine) is much more prolonged than that of *l*-hyoscyamine, but is definitely separated into two parts. For the first twenty-four to thirty-six hours after the injection of either of these isomers into the frog, the symptoms are very similar, consisting of pure curara action on the terminations of the motor nerves. Later the animal under *l*-hyoscyamine recovers without further complications. The atropine animal recovers from the peripheral action only to show a new strychnine-like stage of increased reflex and tetanic convulsions, which may persist for several days after the *l*-hyoscyamine frog has completely recovered. In an earlier paper (12), I showed that this was due to the action of *d*-hyoscyamine which induces first paralysis of the peripheral motor nerves, and in a later stage stimulation of the spinal cord. At that time I had only enough *d*-hyoscyamine for one experiment on the frog, and having now a larger supply kindly sent me by Professor Pyman, I have repeated the observation with the same result.

In the initial stages of partial curara action, I observed no difference in the behaviour of frogs under similar doses of *l*- and *d*-hyoscyamine. The muscular weakness and incoördination developed in equal degree and rate. But when, after twenty-four hours or longer, the curara action began to pass off, the recovery of the animal under the *d*-hyoscyamine was distinctly slower than that under the *l*-isomer. The latter soon recovered completely, while the *d*-hyoscyamine frog showed a complex of peripheral paralysis with central stimulation manifested in increased reflex and spasm. The curara effect continued to become less marked, while the strychnine-like action developed, and finally the animal was thrown into tetanic convulsions by the slightest touch or by jarring the table, while the curara symptoms had disappeared completely. This stage of hyperexcitability of the spinal cord lasted for several days and the animal generally recovered.

In a series of experiments doses varying from twenty to one milligram of the two hyoscyamines were compared; at the higher doses the symptoms were those described already—a pure curara action from the *l*-hyoscyamine, pure curara action at first from the *d*-hyoscyamine, then a mixture of curara and strychnine symptoms and finally pure strychnine tetanus. As the dose was reduced to about three to five mgm. in 20 gram frogs, the curara action became less marked from each alkaloid in the beginning; at the end of sixteen to twenty-four hours the frog with *l*-hyoscyamine had recovered completely and remained normal, while that poisoned with dextrorotary still showed symptoms of peripheral action but no symptoms of central stimulation. A few hours later—twenty to twenty-eight hours—it had also recovered completely to all appearances; spontaneous movements were normal and the reflexes were not increased. Within the next twelve hours, however, a new phase set in of increased reflex excitability, which in one experiment culminated in typical tetanic spasms, from which the animal recovered in about seventy hours from the time at which the reflex stimulation set in. Smaller doses of *d*-hyoscyamine (2 mgm.) caused slight peripheral impairment, with clumsiness in movement and early fatigue, but no distinct rise in the reflex excitability. Finally 1 mgm. had no apparent effect whatever. The two isomeric hyoscyamines thus differ in the facts that (1) the *d*-hyoscyamine has a more prolonged action on the peripheral motor nerve terminations, which suggests that it is more slowly destroyed in the frog tissues than the natural laevorotary isomer; and (2) that it induces a second phase of increased reflex excitability and tetanus, which is absent under *l*-hyoscyamine. This phase may occur after the first curara-like action has passed off and the animal has apparently recovered completely; it probably does not arise from the *d*-hyoscyamine itself but from some decomposition product, which still remains in the tissues.

The general action of the hyoscines on mammals was examined by making hypodermic injections of the two isomers in rats and mice. But the former were found too resistant to the alkaloids to be available. Thus two rats of 120 grams and 110 grams weight

received 20 mgm. of *l*-hyoscine and *d*-hyoscine hydrobromide respectively, but showed no definite symptoms in two and a half hours; 40 mgm. additional hydrobromide was then injected into each, but no effects were observed in either rat. The lowest effective dose of either hyoscine for the rat is therefore more than 0.3 mgm. per gram. The lowest effective dose in man is given in the pharmacopoeias as 0.3 mgm., presumably for a patient of about 70 kgm.

Sixteen mice were injected in pairs with the two hyoscine hydrobromides and were also found to possess a high degree of tolerance for them. About one mgm. per gram body weight seemed to cause slight depression, but definite symptoms could hardly be made out from less than 1.5 mgm. per gram. Smith states that the minimal lethal dose was 1.3 mgm. per gram for mice of one litter, while for those of another it was 0.7 mgm. per gram. These are much smaller doses than I found effective; it is essential in these experiments to keep the animals warm, since mice lose heat very rapidly.

The first effects were manifested in lessened spontaneous movements, the animal moving about less and the head sinking towards the table until it rested on the lower jaw. Movements of escape could be elicited readily by touch or by jarring the table and spontaneous movements occurred at intervals. These movements were accompanied by coarse tremor of the muscles, which was absent when the animal lay still, and which reminded one of the coarse tremor seen in the partially curarised frog. The hind legs were often stretched out behind the animal, as if they were paralysed. The respiration was generally slow and deep at this stage. Not infrequently a quick twitching movement occurred. The mouse reacted with movements of escape when the tail was pinched. Rather later all movement ceased except the respiration; the animal could be laid on its side or back without resistance. The respiration became slower, then weaker, finally occasional, and then stopped. No difference could be made out between the effects of the two bases. In one pair of experiments there may be more tremor in movement or more frequent twitching from one hyoscine than from the other, but in the next pair the symp-

toms may be reversed. The fatal dose in mice varies in very large limits as is seen in the table below, in which the fatal doses are indicated by †.

LAevo-HYOSCINE	DExTRO-HYOSCINE
<i>mgm. per gram</i>	<i>mgm. per gram</i>
0.42	0.38
0.95	0.95
1.6†	1.8†
1.4	1.4
1.65†	1.65
1.85	1.85
2.0	2.0
3.0	3.0†

Here 1.6 mgm. per gram was fatal to both mice, while all recovered from 1.85 and 2.0 mgm. and one from even 3 mgm. per gram. This variation may arise from the rapid destruction or excretion of the hyoscines. It was very striking how in some instances a mouse that seemed dying, recovered and was fairly normal in an hour or less. The minimal fatal dose for these mice is higher than that found in earlier experiments with Peebles, perhaps because in the latter no precautions were taken to maintain a constant temperature. In the experiments which proved fatal, the symptoms came on early and progressed rapidly, while in others in which larger doses were injected, the symptoms were delayed and the animal recovered. The cages were kept warm throughout, so that the variation cannot be ascribed to differences in the temperature. And the solutions amounting to about 0.4 cc. were warmed before injection. I ascribe the variation in the fatal dose to differences in the rate of absorption. These variations were so great that it did not seem promising to continue the experiments, especially as my supply of *d*-hyoscine was limited. As far as they go, they fail to indicate any definite difference between the two isomers in symptoms or lethal dose in mice. These animals possess a remarkable tolerance for hyoscine, some surviving a dose of 3.0 mgm. per gram. This would correspond to 200 grams in man, while fatalities are recorded from 1 mgm. I am sorry that I have been unable to obtain observations of the

relative sedative effects on the brain of these isomers. These must be made clinically, and I have not found any one prepared to undertake the comparison so far.

These experiments thus confirm the results obtained by us previously. The "partially racemised" natural hyoscine (*l*-troyl-*dl*-oscine) acts fifteen to eighteen times as strongly as the corresponding artificial form (*d*-troyl-*dl*-oscine) on the salivary glands and the mechanism of the intestine involved in the pilocarpine effect, and it may be inferred that a similar ratio holds for the other peripheral neuro-muscular apparatus affected by the group of atropine and hyoscine. The dextro-hyoscine undergoes destruction in the frog more slowly than the laevorotary isomer, indicating that the cells involved in this process are capable of distinguishing between the isomers. The myoneural receptors of striated muscle, smooth muscle, and the central nervous system react equally to the two isomers.

It is to be noted that neither of the isomers under examination was a chemical individual. The *l*-hyoscine contained equal quantities of *l*-troyl-*l*-oscine and *l*-troyl-*d*-oscine, while the *d*-hyoscine was similarly constituted of *d*-troyl-*d*-oscine and *d*-troyl-*l*-oscine. They differed only in the activity of the troyl component while the oscine in each was racemic. It is probable that the individual components of each pair also vary in activity; for example *l*-troyl-*l*-oscine may be found the most powerful of the group, while *d*-troyl-*d*-oscine may be almost devoid of activity. It is at any rate certain that the direction of rotation of the troyl component exercises a dominant influence on the peripheral action and the rate of destruction of the hyoscines. It is impossible to estimate the influence of the oscine component at present.

It may be of interest to put on record the tissues which have thus far been shown to react differently to optically isomeric substances. This power of differentiation is possessed by those cells which destroy the isomers, as was shown by Pasteur for the moulds and by many subsequent observers for various bacteria (see Hirsch (13)). Similarly in the cases of certain sugars and other bodies the mammalian tissues oxidise one isomer readily, while they attack its opposite with difficulty and slowly. The

hyoscines and hyoscyamines differ in the same way in the frogs' tissues, the dextrorotary forms undergoing destruction more slowly than the laevorotary. In some of these instances the selective agent has been definitely shown to be of enzyme nature and in all this is very probable.

Among cells with a more specialised function, it has been shown that the taste organs differentiate between the isomers: e.g., *d*-asparagin, *d*-glutaminic acid and *d*-histidine possess definite tastes, while the *l*-forms have much less effect on the nerve ends in the tongue.

The sympathetic myoneural junctions have proved to react differently to the adrenalines, the *l*-form being much more powerful than the *d*-. The peripheral receptors which react specifically to atropine, whatever their nature, are affected more strongly by *l*-hyoscyamine and *l*-hyoscine than by the *d*-isomers. The central nervous system is stimulated in the frog by *d*-hyoscyamine and not by *l*-hyoscyamine, and *d*-camphor is said by Langgaard and Maas (14) to stimulate it more than *l*-rotary.

In most instances it has been shown definitely that while the oxidising tissues or ferments act preferentially upon one isomer, the selection is not absolute, for both isomers are affected though in different degrees; and similarly though an alkaloid may act many times more strongly than its isomer of opposite sign, the latter also possesses some action. This slight effect of the less active agent suggested that possibly some of it became racemic in the tissues and that the trace of activity was due to the small amount of its antipodal body formed. But it has been shown (11) that the characteristic action of atropine or hyoscyamine is shared by a large number of tropine compounds which have no asymmetric carbon atom; the asymmetry of atropine and hyoscyamine does not endow the molecule with any new powers, but merely intensifies those present in allied but optically inactive compounds. The action of these cannot be attributed to the formation of optically active substances and similarly that of *d*-hyoscyamine is inherent in it and not derived from *l*-hyoscyamine formed in the tissues.

Asymmetric carbon has no specific action in itself, and two laevorotary substances of different constitution need have nothing in common in their action (e.g., adrenaline and hyoscyamine), because the point of action is determined not by the asymmetry and direction of rotation but by some unknown property of the molecules. The asymmetric carbon has an intensifying action, but does not determine which cells react to the molecule. In some instances this intensifying effect is absent, even when there is definite action elicited by the poison; for example, the myoneural receptors of the striated muscle of the frog are paralysed by both hyoscyamines and both hyoscines with equal intensity, though the action lasts longer in the case of the dextrorotary alkaloids probably owing to their slower destruction in the tissues. Similarly the augmentor and depressor action on smooth muscle is the same whichever isomer is employed, and this is in contrast with the antagonistic action on the smooth muscle. This indicates a fundamental difference in the character of the effects of such an alkaloid as atropine; some of these effects are increased by the presence of asymmetric carbon and the direction of optical rotation, while others are unaffected. In the present state of knowledge the former effects appear to be due to the physical properties of a chemical compound formed between an optically active receptor substance and the alkaloid (11); the second series of effects do not necessarily imply the formation of a chemical compound between tissues and poison, and may arise from the physical properties of the unaltered alkaloid. This view seems to be strengthened by the fact that the actions which vary with the direction of rotation are the so-called "specific" actions of the group, that is those which are confined to a small number of substances which are nearly related in chemical constitution and in which some common chemical properties may very well reside. The actions which are unaffected by the direction of optical rotation on the other hand, of which the curara-like effect in the frog may be taken as a type, are common to a very large number of substances which are of dissimilar character chemically and of which it is difficult to expect a similar chemical reaction in the tissues.

SUMMARY

1. Two hyoscines, each of them racemised in the oscine component, but opposed in the direction of rotation of the tropyl radicle, were examined and it was found that the laevorotary hyoscine is fifteen to eighteen times as powerful as the dextro-rotary in action on the terminations of the nerves in the salivary glands and in other "specific" atropine effects.

2. *D*-hyoscine and *d*-hyoscyamine are more slowly destroyed in the tissues than *l*-hyoscine and *l*-hyoscyamine.

3. The action of the hyoscines on the nerve ends in striated muscle, on unstriated muscle, and on the central nervous system is identical.

4. Similar results were obtained in comparing the two hyoscyamines, except that *d*-hyoscyamine possesses a late stimulant effect on the spinal cord, which may be ascribed to the presence of some decomposition product rather than to the alkaloid itself.

5. It is suggested that the "specific" effects of the atropine group arise from the physical properties of some chemical compound formed with an optically active substance in the tissues, while the less specific effects may be explained by the properties of the uncombined alkaloid.

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ASTRINGENCY AND PROTEIN-PRECIPIRATION BY MASKED TANNIN COMPOUNDS¹

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INTRODUCTION

The use of tannins in diarrhea has led to the introduction of a number of compounds that are intended to avoid any action in the stomach, and to develop astringent effects in the intestine. In principle, these are based on the differences in reaction (hydrogen-ion concentration) that prevail at the different levels in the alimentary tract; i.e., the preparations are intended to be insoluble in the acid stomach, but to dissolve and become astringent in the intestines.

These properties are claimed, at least by inference, for numerous commercial tannin compounds.

With the hope of arriving at an independent and objective classification of these agents, it appeared worth while to check up the data as to solubility and as to astringency, under uniform conditions that would simulate those of the digestive tract, at least as to the degree of reaction (hydrogen-ion concentration) and time of contact.

The solubility determinations were undertaken by Dr. P. N. Leech. His results are being published in detail elsewhere; but they have such important relations to astringency, that brief abstracts to them will be given in this paper.

However, the solubility of a tannin-compound in itself does not guarantee astringency. It is necessary that the solution

¹ This investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

contain the tannin free, or in such a form that it may react with proteins. This can only be known by the direct experiments which form the main portion of this investigation.

There are several methods of demonstrating astringency, by the precipitation of proteins or on excised tissues, or on living animals. As the relative value of these was not known, several methods were tried, including a new modification, viz., anti-hemolysis by tanning red corpuscles. These, and the results obtained with them, will be described seriatim.

Method of determining the solubility. Leech investigated the solubility under conditions approximating those of the body; i.e., by digestion at 40°C. for three and one-half hours, with frequent agitation. The undissolved residue was then collected on a filter, dried, and weighed. One gram of the tannin-preparation was used for 250 cc. of solvent. The solvents were:

Water.

Dilute acid: $N / 12.5$ (0.2 per cent) HCl.

Acid-pepsin: This acid, with 0.1 per cent of pepsin.

Alkaline: Sodium bicarbonate 1 and 2 per cent. These gave practically identical results.

Alkaline trypsin: Bicarbonate with 0.05 per cent of holadin.

Results of solubility determinations. These are summarized in table 1.

All the commercial masked tannin compounds depart more or less in solubility from the claims made for them; and there are quite wide variations in different specimens of the same product.

Solubility in water: None are insoluble, or even "practically insoluble" in water. This, however, has probably but little therapeutic importance.

Solubility in dilute acid: Acetannin comes nearest to being insoluble in the acidity of the gastric juice. All the other compounds dissolve fairly readily.

The presence of pepsin increases the acid-solubility of tannin albuminate materially, presumably by dissolving the heat-coagulated albumen. With the other compounds, pepsin either has no effect or the solubility *appears* decreased through the tannin-precipitation of the protein of the pepsin.

Insolubility in the gastric juice would be desirable, theoretically, by precluding undesired gastric effects. In fact, however, it is probably not important, provided that the solution is slow, or if the tannin is taken with food.

The solubility in bicarbonate varies quite widely. With the albumen-tannates, it is materially aided by trypsin. The speed of solution, and the liberation of tannin from its esters, are also factors of practical therapeutic importance.

Rapid solution and liberation of the tannin would be favorable to intensive but short action in the upper intestine; whereas slow solution or liberation would be necessary for prolonging the action into the lower intestines.

TABLE 1

Solubility (range and median) of masked tannin compounds

TYPE	WATER	HCl	HCl WITH PEPSIN	BICARBONATE	BICARBONATE WITH HOLADIN
Tannalbin.....	18-22 (21)	32-34 (33)	56-87 (75)	41-52 (42)	45-68 (58)
Albutannin.....	15-16 (16)		95-98 (96)	31-34 (33)	77-86 (82).
Protan.....	14-16 (16)	30-38 (35)	73-81 (77)	90	81-98 (94)
Acetannin and tannigen.....	6.1-17 (8.5)	(6.2)	3.8-17.4 (5.8)	31-100 (82)	32-100 (76)
Tannoform.....	21-47 (32)	32-42 (37)	12-21 (15)	81-95 (87)	78-96 (84)
Tannopin.....	17	32	20	26	24
Tannismuth....	18	25	15	15	13
Gallogen.....	18	6.2	16	15	17

From this standpoint, the compounds would therefore fall into the following groups:

a. Compounds easily soluble in reactive form, and therefore likely to exhaust their action in the upper intestine: Protan.

b. Compounds with protracted action because slowly soluble: Albutannin.

c. Compounds with protracted action because slowly hydrolyzed: Acetannin.

d. Compounds that are less than half soluble. They are probably mixtures, and it would be difficult to predict their actions from their solubility: Tannoform, tannopin, tannismuth, gallogen.

THE PRECIPITANT ACTION OF MASKED TANNIN COMPOUNDS ON
EGG-ALBUMEN

It has been shown that these compounds are, for the most part soluble in acid, and usually somewhat more soluble in alkaline media. The next question was, whether these solutions were able to precipitate proteins at reactions corresponding to the acidity of the stomach and the alkalinity of the intestines. One method of approaching this was by estimating the diminution of protein-nitrogen when they were left in contact with an egg-albumen solution. This constitutes a good method for comparing the products. The experiments were carried out by O. H. Schettler and N. C. Wetzel.

Method. The albumen was used in 5 per cent solution of the moist egg-white in $N 7 / 100$ HCl and in 1 per cent sodium bicarbonate, respectively. To 50 cc. of these there was added 1 gram of the tannin drug. The mixture was set aside with frequent shaking, for twenty-four hours, centrifuged, and the nitrogen determined by the Kjeldahl method in duplicate samples of 5 cc. of the clear fluids. In the albumen solutions, without tannins, the nitrogen amounted to 6.2 to 6.8 mgm.

In the case of the various brands of tannalbin, the drug was also macerated without the egg-albumen, and the nitrogen of its solution determined.

Each series was carried through as a single operation.

Results with egg-albumen precipitation. These are compared in tables 2 and 3. They show that:

The tannin albuminates alone precipitate albumen in acid solution. This indicates that they have least perfectly attained the therapeutic object of avoiding precipitation in the stomach.

All the classes precipitate albumen in alkaline solution; the individual samples varying quite as much as the classes.

The tannigen class represents most perfectly the theoretical therapeutic objects of non-precipitation in acid reaction (stomach) and extensive precipitation in alkaline reaction (intestines).

Tannoform, tannopin and gallogen, however, also fulfill these objects.

The protein of commercial tannin albuminates is somewhat soluble in both acid and alkaline media, and to about the same degree.

TABLE 2
Percentage of albumen precipitated by tannins

	HCl N 7/100	SODIUM BICARBONATE 1 PER CENT
Tannin.....	63	100
Tannigen (1)*.....	0	62
Acetannal.....	0	96
Tannalbin (2)*.....	26	76
Tannin Alb Exsic (24)*.....	26	39(?)
Albutannin (Protannal).....	35	97
Tannoform (4)*.....	0	57
Tannopin (12)*.....	0	50
Gallogen (11)*.....	0	58

* These are the code-numbers of the specimens. A detailed list of these is given in Lecch's paper.

TABLE 3
Solubility of the proteins of commercial tannin albuminate. In terms of milligrams of nitrogen dissolved per 100 mgm. of drug

	HCl N 7/100	SODIUM BICARBONATE 1 PER CENT
Tannalbin (2).....	2.0	1.6
Tannin Alb Exsic (24).....	2.0	0(?)
Protannal.....	2.3	3.8

SERUM PRECIPITATION AS A CRITERION FOR ASTRINGENT ACTION
OF MASKED TANNINS

In as much as astringency is probably proportional to protein precipitation, any convenient protein should be suitable for comparative tests. In this series, a sample of dried serum was used, as it permitted the making of solutions of uniform concentrations at different time. The experiments were planned to illustrate the influence of various reactions, concentrations, etc.; and were generally combined with hemolytic and other tests, to determine the practical value of the different methods. The relations will be brought out later.

General course of experiments. The typical experiments consisted in placing into numbered test-tubes 10 cc. of the *solvents* (see below); then the weighed quantity of the astringent drug. The tubes were then stoppered, shaken and digested at 40°C. for four hours, inverting the tubes every hour. At the end of this time, these *tannin digests* were filtered. The taste of the solution was noted for astringency. Then 5 cc. of the filtrate were made neutral to methyl orange by HCl and bicarbonate (i.e., pH = 3.1 to 4.4), and added to 5 cc. of the *serum solution* (see below).

The occurrence of precipitation was observed, at once, and again after one to three days. Observations were also made on the solubility of some of the precipitates in sodium bicarbonate.

The following solvents were used:

I. "NaCl;" 0.9 per cent (i.e., isotonic saline).

II. "HCl;" 7 cc. of normal in 100 cc. of 0.9 per cent NaCl. This corresponds to about 0.25 per cent of HCl, and to pH = about 1.16. It was intended to represent the acidity of the gastric juice.

III. "Pepsin;" 0.25 per cent of pepsin in "HCl" II; intended to represent gastric juice.

IV. "Bicarb;" Sodium bicarbonate 0.5 per cent in NaCl 0.9 per cent; representing the alkalinity of the intestine.

V. "Trypsin;" 0.25 per cent of trypsin in "Bicarb" IV; representing pancreatic juice.

VI. "Bicarb-bile," bile salts (bilein) 0.063 per cent in "Bicarb" IV; representing the bile.

VII. "Carb," sodium carbonate, 1 per cent.

Serum solution. This was made by rubbing 2.5 gram of dried and powdered serum in a mortar with 0.9 per cent NaCl to a total of 250 cc. It was left to stand half an hour, and filtered through filter paper.

Results. These will be discussed later, under the individual drugs.

BLOOD-CORPUSCLE METHOD OF TESTING ASTRINGENCY²

The precipitation of proteins in cell membranes is assumed to render these less permeable, analogous to tanning. Accordingly

² About eighteen months after these experiments were completed, I find that Kobert (1918) has also published a "blood-corpuscle method" for the quantitative evaluation of astringency. His method, however, is quite different from that used by me for it measures the agglutinating power, and not the protection against hemolysis.

it was to be expected that treatment of red blood corpuscles with astringent solutions would render them resistant to the laking action of water; i.e., that astringency would result in anti-hemolysis. This supposition was confirmed by experiment. When a 1:10 dilution of defibrinated dog's or sheep-blood in 0.9 per cent NaCl is added to a 1 per cent tannin (1:10) solution in 0.9 per cent NaCl, a fawn colored precipitate of corpuscles is formed. This is allowed to stand for five minutes. Water may then be added (10:1) without the occurrence of laking, even after four days.

Threshold concentration of tannin. 0.25 per cent was found perfectly effective; 0.0625 per cent was doubtful.

Laking by solvents. The solvents used in the digestion experiments were tested on the corpuscles by a similar technic. HCl N / 15 produced direct laking when added to the blood suspension; but not, of course, if the acid was first neutralized. Acid digests were therefore neutralized before testing. Sodium bicarbonate, 0.5 per cent, does not produce laking. Bile-salts would produce laking and therefore were not used in making digests.

Antihemolysis by masked tannin compounds in various media. This was investigated in several series of experiments. Since they differ only in minor details, they may be discussed together.

General method. The subsequent experiments were carried out according to the following method.

Tannin digests. These were prepared as explained under serum precipitation. In fact, the same digests were used in some of the experiments, thus permitting the direct comparison of the two methods.

All solutions contained 0.9 per cent NaCl.

Blood suspension. 10 per cent of defibrinated dog's or sheep's blood in 0.9 per cent NaCl.

Mixture. The digests were filtered and used either directly or after neutralization. To 5 cc. of the digest was added 0.5 cc. of the blood suspension, the mixture set aside for half an hour, and *direct laking* observed.

Two cubic centimeters of the mixture was then added to 20 cc. of water, set aside, and observed for *water laking*.

Results. These will be discussed under the individual drugs.

Astringent taste of solutions

Notwithstanding its subjective nature, the taste is quite a fair index of astringency. In a 0.9 per cent NaCl solution of tannin the threshold of taste and of protein precipitation coincide closely, as shown by the following:

PER CENT OF TANNIN	TASTE	PRECIPITATION OF SERUM
0.0625	Slightly astringent	Turbidity
0.03125	Slightly astringent	Doubtful
0.015625	Not astringent	None

The filtered digests made of 2.5 per cent of tannalbin, tannigen and tannoform respectively were tested with the following essentially negative results, which agree throughout with the serum precipitation.

NaCl. Taste not astringent with any.

Pepsin-HCl, neutralized. Doubtful with tannalbin; not astringent with the others.

Trypsin-Bicarb, neutralized. Doubtful with all.

Astringent taste of the dry powders

The taste of the dry powders seems to be a more delicate test of astringency than the taste of the solutions. A penknife point-full of the powder is placed on the tip of the tongue and chewed. With this test, the following results were obtained, in ascending order of astringency. The bracketed numbers are the code numbers of the specimens.

Tannopin (12 and D): Entirely non-astringent.

Tannigen (1, 6, 13, 25) and Acetannal: Acid; then slightly astringent.

Gallogen (11, 19 and 22): First slightly salty and acid; then slightly astringent.

Tannalbin (A, 2, 9, 14) and Tannin Albuminate exs. Merck (17, 24): Slightly astringent.

Tannismuth (10, 20): Quite astringent from start.

Protan (5, 7, 16, 23): Quite astringent from start.

Tannoform (3, 4, 8, 15): Quite astringent from start.

Tannigen (exceptional no. 21): Highly astringent. Evidently decomposed.

The frog-lung method of estimating astringency

Dresler suggested the extensibility of the frog-lung as an index of astringency. It can be measured by tying the sacular lung over a cannula. This is attached to a tube, which is immersed to a given depth in a cylinder of water. The level to which the water rises in the tube is proportional to the expansion of the lung. It is diminished by laying the lung into astringents. The details are described in Sollmann, Laboratory Guide, page 159. Comparisons may be made by working under standard conditions.

The following experiments were carried out by Mr. G. E. Richardson. For each reading, the tube was immersed in the cylinder for one-half minute. The lung was then laid into the astringent solutions for the periods shown in the experiments. All solutions were made up with 0.6 per cent NaCl. Acid and bicarbonate both corrode the lungs; so that the digests were neutralized before testing.

Results. These are shown in table 4. The figures correspond to the differences in per cent of the original reading in the same experiment. “+” indicates increase of extensibility, “-” decrease of extensibility, i.e., astringent action. Only the median figures for all experiments are reproduced.

It may be noted that in saline alone, the extensibility of the lung tends to increase (till about forty-five minutes); but in all of the astringents, it diminishes. Typical astringents, viz., copper sulphate and tannic acid, cause a rapid and profound decrease of extension. This tanning action begins at once and is well advanced in five minutes. In the case of tannic acid, it is usually completed in one-half hour. The effect is proportional to the concentration.

As to the masked tannin-compounds, neutral saline extracts contain sufficient tannin to be very distinctly astringent. Extracts made with artificial gastric juice are still more astringent. The results for alkaline tryptic digests are probably unreliable.

The results leave no doubt that these preparations are astringent for tissues, and as potent as tannin solutions of 0.5 per cent or stronger. In vivo the degree of astringency would probably be less, because of the relatively slow solubility. On the other hand, this would prolong the action farther into the intestines.

TABLE 4
Median effects of astringents on frog-lung

	APPROXIMATE TIME OF IMMERSION OF LUNG IN ASTRINGENT			
	5 minutes	15 minutes	30 minutes	$\frac{3}{4}$ to 1 $\frac{1}{2}$ hours
<i>A: Astringents in 0.6 per cent NaCl</i>				
None (i.e., NaCl alone).....		+4	+26	+28
CuSO ₄ 0.1 per cent.....	-55	-58	-74	
Tannic acid 2.5 per cent.....		-59	-70	-87
Tannic acid 1 per cent.....	-11	-23	-48	-48
Tannic acid 0.5 per cent.....		-30	-35	-62
Tannic acid 0.25 per cent.....		-18 $\frac{1}{2}$	-23	-33
Tannalbin, Spec. 2, 2.5 per cent.....		-46	-58	-64
Tannigen, Spec. 1, 2.5 per cent.....		-22	-40	-40
Tannoform, Spec. 3, 2.5 per cent.....		-30	-24	-52
Tannalbin, Spec. A, 1 per cent.....		-15		
Tannigen, Spec. B, 1 per cent.....		-20		
Tannoform, Spec. C, 1 per cent.....		-3	-14	-26
Tannopin, Spec. D, 1 per cent.....		-5	-10	-18
<i>B: Acid-pepsin digests, neutralized to litmus:</i>				
None (i.e., NaCl alone).....		-7	-19	-30
Tannin, 2.5 per cent.....		-75	-78	
Tannalbin, Spec. 2, 2.5 per cent.....		-37	-48	-67
Tannigen, Spec. 1, 2.5 per cent.....		-33	-55	-77
Tannoform, Spec. 3, 2.5 per cent.....		-40	-84	-84
<i>C: Bicarbonate-trypsin digests, neutralized to litmus:</i>				
None (i.e., NaCl alone).....		+5	+5	+5
Tannin, 2.5 per cent.....		-13	-63	-63
Tannalbin, Spec. 2, 2.5 per cent.....		-4	-3	-22
Tannigen, Spec. 1, 2.5 per cent.....		+1	+5	-2
Tannoform, Spec. 3, 2.5 per cent.....		+1	+1	-5

The lung-test is evidently a good qualitative and rough quantitative test of astringency, but it is much less convenient than the protein precipitation test, and is unnecessary except as a control for special questions.

TANNIN PREPARATIONS ON NORMAL STOOLS

Some experiments were performed on a normal human subject to note whether tannin astringents have a constipative action on normal stools. The experiments were made as a continuous series, and the results are given in the sequence in which they were obtained.

	<i>Average number of stools of day</i>
<i>Fore period</i> , 4 days.....	1
<i>Gallic acid</i> , 1 gram in milk, after lunch	
Two following days.....	1
<i>Tannin</i> , 0.5 gram in milk, after lunch	
Next day.....	1
<i>Tannin</i> , 2 grams in tea, with lunch	
Next day.....	1
Second to ninth day.....	1½
Tenth and eleventh day.....	1
<i>Catechu</i> , 3 grams, powdered, in tea, with lunch	
Next day.....	1
<i>Catechu</i> , 10 grams, powdered, in tea, with lunch	
Next day.....	1
Second to seventh day.....	2
Eighth and ninth day.....	1½
<i>Tannalbin</i> (A) 10 grams, dry, after lunch	
Next day.....	2
Second and third day.....	1

It is very evident that there is no effect on the number of normal stools; nor was the normal formed consistence affected. The experiments were not continued further. Determinations of the moist or dry weight might have been more positive, but it seemed improbable, in view of these negative results, that they would justify that trouble of exact control of the diet.

The astringent effects in the mouth and esophagus were interesting, as to their intensity and especially as to their duration. The suppression of secretion persisted over half an hour, indicating that the tannin-tissue precipitations are but slowly dissolved. The details are as follows:

Tannin, 2 grams, in 0.5 liter of tea, swallowed slowly with lunch. Taste very distinctly astringent; throat dry and swallowing difficult. The dysphagia lasts about half an hour. During the afternoon there is some heart-burn and heavy gastric sensation.

Tannin, 0.5 gram in milk: Effects not marked.

Gallic acid, 1 gram in milk: Taste slightly bitter; not astringent, except the dregs.

Catechu, powder, 3 grams and 10 grams, in tea: Astringent taste. Throat slightly dry with the smaller dose, distinctly dry with the larger dose.

DISCUSSION OF THE RESULTS WITH THE INDIVIDUAL DRUGS

In this section there will be discussed the results obtained on the individual drugs, with the various methods. The percentages of the drugs are stated in terms of the final mixtures. Unless otherwise explained, "neutral saline" stands for 0.9 per cent NaCl, except in the frog-lung experiment, where 0.6 per cent NaCl was used. "Acid digest" means the filtrate after about four hours digestion with saline containing $N/15$ HCl, i.e., about the acidity of gastric juice. "Bicarbonate digest" means 0.5 per cent sodium bicarbonate under analogous conditions; i.e., the reaction of the intestinal juice. "Neutral" means that the filtrate was neutralized to methyl orange; except with "sodium carbonate," which was digested with 1 per cent of the carbonate and the filtrate neutralized to litmus. The further details were described under the methods.

Tannic acid

This serves as a standard of comparison for the others.

Albumen precipitation. When 2 per cent of tannin was added to 10 per cent moist egg white, the albumen was completely precipitated in 1 per cent sodium bicarbonate solution; 63 per cent was precipitated in $N/15$ HCl. Precipitation therefore occurs at full gastric acidity, but is less complete than when the solution is less acid.

Tannin and serum

Neutral saline tannin digest. Precipitation of serum begins with mixtures containing 0.015625 per cent of tannin, and is complete with those containing 0.125 per cent of tannin. The precipitate is much greater than with any of the tannin compounds.

The following serum experiments all refer to mixtures containing 0.125 per cent of tannin.

Acid digest. Before neutralization, this digest produced only turbidity, when added to serum. After neutralization, the turbidity was very much heavier.

Acid-pepsin digest. Before neutralization, the digest did not precipitate serum; but after neutralization, it gave a heavy precipitate. The failure to produce turbidity in acid reaction is probably due to the binding of some of the tannin by the protein of the pepsin.

Bicarbonate digest. Before neutralization, serum produced only turbidity; after neutralization, it gave a strong precipitation.

Tannin acid destruction by sodium bicarbonate. If the tannin is digested for several hours at 40°C. with 1 per cent sodium bicarbonate, and then neutralized, distinctly higher concentrations are needed for precipitation; than with neutral saline digests, viz., 0.0625 and 0.5 per cent for beginning and complete precipitation; instead of 0.015625 and 0.125 per cent respectively. This indicates that some of the tannin is destroyed in alkaline digestion experiments.

Trypsin-bicarbonate digest. This behaved essentially like the bicarbonate; both before and after neutralization. The precipitate in the neutral solution was not quite as heavy, presumably because some of the tannin had been precipitated by the protein of the trypsin.

Bile-bicarbonate digest. This gave precipitation even without neutralization.

Sodium carbonate digest. After neutralization, this gave a precipitate showing that the tannin had not been destroyed.

Antihemolytic efficiency of tannin digest

Saline solutions. A concentration of 0.125 per cent protects completely against water-laking. With 0.0625 per cent, the protection is partial. With 0.03125 per cent, there is practically no protection. (Kobert found complete agglutination with 1:20,000.)

Acid digest and acid-pepsin digest. 0.1 per cent tannin, after neutralization protects completely.

Bicarbonate digest. Before neutralization, 0.125 per cent protects only incompletely. After neutralization 0.1 per cent gives complete protection.

Trypsin-bicarbonate digest. Before neutralization, no protection with 0.25 per cent; after neutralization, complete protection with 0.25 per cent.

Sodium carbonate digest. After neutralization, 0.25 per cent gives complete protection.

Astringent taste of saline tanin solution

Slight astringency is noted with 0.03125 per cent; none with 0.015625 per cent.

Tannin on lung elasticity

The expansion is diminished 23 per cent by 0.25 per cent tannin, and 74 per cent by 2.5 per cent tannic acid. Practically the same results are obtained with the neutralized acid-digest.

Summary of tannic acid

The various methods of testing astringency confirm that tannin is astringent for the whole range of reactions that exist in the digestive tract; but that the precipitation, and therefore the astringency are less efficient at the extremes of reaction; the extreme acidity or the gastric juice being more unfavorable than the alkalinity of the intestinal juice.

The individual methods compare as to sensitiveness as follows:

	BEGINNING REACTION	MARKED REACTION	COMPLETE REACTION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Per cent of tannin needed for:			
Serum precipitation.....	0.015625		0.125
Antihemolysis.....	0.0625-0.03125		0.125
Taste.....	0.015625-0.03125		
Lung expansion		0.25	

Acid digests. Before neutralization, these produce only slight precipitation of serum and incomplete precipitation of egg-albumen.

Acid digests after neutralization. These produce full-strength reactions by all the methods.

Pepsin-acid digests. These are slightly weaker than the simple acid digests; presumably because a part of the tannin had been bound by the protein of the pepsin.

Bicarbonate digests before neutralization. These give complete precipitation of the egg albumin, but only turbidity with serum, and incomplete protection on laking.

Bicarbonate; trypsin-bicarbonate; and bile-bicarbonate digests after neutralization. These give a strong precipitate with serums and complete protection against laking. However, some of the tannic acid is destroyed during the alkaline digestion.

Sodium carbonate digests. After neutralization, these precipitate serum and protect against hemolysis.

Gallic acid

It was pointed out in the previous article that this precipitates proteins in buffer solutions essentially like a weak tannic acid solution; presumably because it is contaminated with a trace of this substance.

Serum experiments with gallic acid. The present series showed no precipitation in the natural (acid) reaction of 0.5 to 4 per cent mixtures. After neutralization, slight turbidity occurred; also in neutralized acid, acid-pepsin, bicarbonate, and bicarbonate trypsin digests, containing 0.25 per cent of the gallic acid.

Gallic acid and other proteins. Egg-white solution, 1:10, was only rendered slightly turbid. The turbidity was not increased by sodium bicarbonate; nor by acacia. The latter experiment was made to test an old statement, attributed by Headland to Pelletier.

Dog-serum gave a large precipitate; not increased by sodium bicarbonate.

Antihemolytic efficiency of gallic acid. Saline solutions of gallic acid are directly hemolytic, doubtless through the acidity.

After neutralization there is no direct laking; nor is there any protection against water-laking. The same total inefficiency was found for neutralized digests with acid, pepsin acid, bicarbonate, and trypsin-bicarbonate. (Kobert found that saline solutions of gallic acid had one-sixtieth of the agglutinating efficiency of tannic acid.)

Summary of gallic acid. Gallic acid behaves like a very dilute tannic acid, i.e., a 0.5 per cent solution of gallic acid is about equivalent to 0.03 per cent of tannic acid. It would have little or no practical value as an astringent.

TYPES OF MASKED TANNIC COMPOUNDS

These fall into several groups:

Astringent extracts

It has been generally supposed that the colloidal extractives of crude drugs and extracts would slow the access and action of their tannin, so as to avoid gastric effects, and would therefore prolong the action into the intestines. Direct experiments (Sollmann, l.c.) show, however, that solutions of these drugs precipitate like tannin itself. Delay in the action could occur by delay in the solution. Such delay would be probable in the case of the powdered drugs, and perhaps with the solid extracts; but it would not apply to solutions, infusions, etc.

It is conceivable that the astringent action would be slowed by the colloids impeding the access of the tannin of the mucosa.

TANNIN-PROTEIN COMPOUNDS

The precipitation of proteins by tannin has been utilized to render the tannin itself more or less insoluble, with the intention of checking or delaying its action. As has been explained, such precipitates may be redissolved under suitable conditions in the digestive tract, and thus reacquire astringent properties.

Freshly precipitated tannin albuminate

This was introduced by Lewin, 1881, and again endorsed by him in 1904. It would have relatively little effect on the stomach; although it would be somewhat astringent at the optimal reaction (Sollmann, l.c.) It would become astringent in the duodenum; but could probably not act much beyond this.

Lewin employed a mixture of 2 to 5 grams of tannic acid in 150 cc. of water, to which the white of an egg is added, gradually and with constant stirring.

Coagulated tannin albuminate

This was introduced by Gottlieb, 1896, and was marketed under the name Tannalbin. New and non-official remedies (N. N. R.) has adopted the group-name "Albutannin" for all the commercial brands. These differ, however, in solubility.

Tannalbin is a heat-coagulated tannin-albuminate, made by precipitating egg-albumen with tannin, washing the precipitate, and drying for six hours at 126°C. It should contain about 50 per cent of tannin, and is claimed to be practically insoluble in water; partly soluble in dilute acid, and slowly soluble in alkaline fluids, with liberation of its constituents.

Clinical trials were conducted by O. Vierordt, 1896. He considers it better than tannigen: The taste is but little astringent, even when kept in the mouth for a considerable time. It was satisfactorily efficient in acute and chronic diarrheas. In colitis, it diminished the mucus materially. The action was uniform. There was no gastric disturbance. It did not influence healthy stools.

Subsequent clinical trials have been satisfactory, without establishing any definite superiority over the other "insoluble" tannin preparations.

Solubility of tannin-protein compounds

The determinations of Leech, conducted under conditions approximating those of the body, differ radically from the advertised claims.

The investigation included specimens of the original "Tannalbin" (Knoll); of "Tannin albuminate Exsicated" (Merck); a specimen each of Calco and M. C. W. (Mallinchrodt) "Albutannin;" and also specimens of "Protan" (Mulford), an analogous tannin casein compound.

The median solubility is shown in table 5.

All dissolve to a considerable degree, though slowly, in water. More dissolves in dilute acid, and still more in the presence of pepsin. Bicarbonate generally dissolves but little more than acid, and less than acid-pepsin. Even trypsin-bicarbonate tends to dissolve less than acid-pepsin.

TABLE 5
Solubility of tannin protein compounds

	MEDIAN AND RANGE OF PER CENT OF THE COMPOUND DISSOLVED BY				
	Water	Acid	Acid-pepsin	Bicarbonate	Bicarbonate-trypsin
Tannalbin, claimed.....	Practically insoluble		Partly soluble	50 per cent slowly soluble, with cleavage	
Tannalbin, found.....	20 (18-21)	34	79 (60-86)	49 (42-52)	59 (58-68)
Merck.....	21	32	72 (72-73)	41	48 (45-51)
Calco and M. C. W....	16 (15-16)		96 (95-96)	33 (31-34)	82 (77-86)
Protan, found.....	16 (14-16)	35 (20-38)	97 (73-81)	90	94 (81-98)
Protan, claimed.....	Insoluble	Insoluble		Soluble	

Comparing the different compounds, it is seen that the Merck product agrees practically with the tannalbin, in showing a rather limited solubility, but greater in acid pepsin than in bicarbonate trypsin. The Calco and M. C. W. are more completely soluble in both media; this may be rather a disadvantage, because it would exhaust the tannin in the upper intestine. Protan differs from the albutannins in its high solubility in alkali, even without trypsin. This also is probably rather disadvantageous.

Speed of solution of the tannin-protein compounds. This is usually a rather slow process. This slowness is quite as important as is the absolute solubility, in determining the site of the astringent action. The results of Leech are shown as curves in figures 1 and 2. In both the acid-pepsin and alkaline-pancreatin digests,

the solution is at first fairly rapid. However, with tannalbin and the Merck product, considerable further solution occurs during the next hour or longer. This would prolong the action of the tannin for considerable distances into the intestine. With protan, the solution in alkaline pancreatin is completed so promptly that the conditions for prolonged action would be unfavorable.

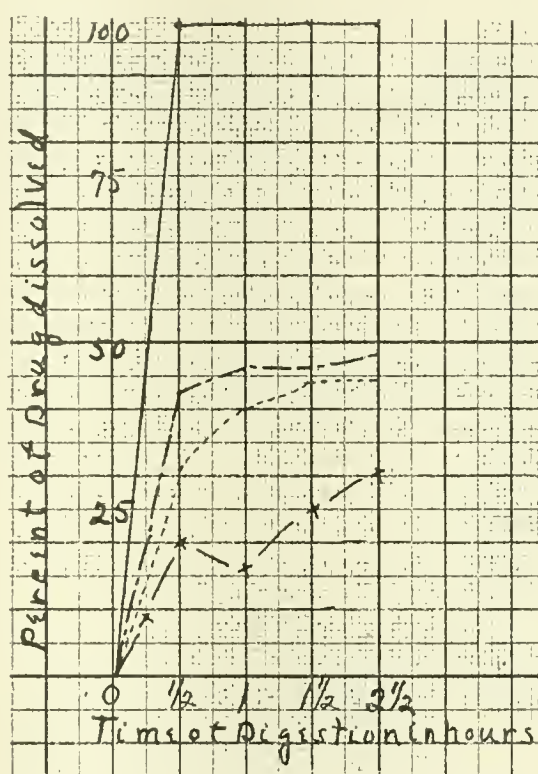


FIG. 1. TIME-FACTOR IN SOLUTION OF TANNIN-PROTEIN COMPOUNDS

Digestion with acid-pepsin. — = protan; = tannin albuminate, Merck; -.-.- = tannalbin; +-+-+ = gallogen.

Is the protein-tannin compound dissolved unchanged? Leech found the nitrogen percentage of the undissolved residue of the Merck product and of Protan after various periods of peptic and pancreatic digestion, as practically unchanged. This indicates that at least the major part of the tannin is really combined with the protein.

Since "peptone" reacts with tannin practically like native proteins (Sollmann; Brühl; Hammersten), the greater solubility of the tannin albuminates in the presence of ferments is probably due

merely to the fact that the ferments facilitate the solution of the heat-coagulated protein-tannin compounds.

Solubility standards. The variation in the solubility of the tannin protein compounds is so wide that it is likely to affect their therapeutic action. It would appear better to restrict the solubility in dilute acid to 25 to 35 per cent; in bicarbonate to 35 to 55 per cent; as determined by the methods employed by Leech.

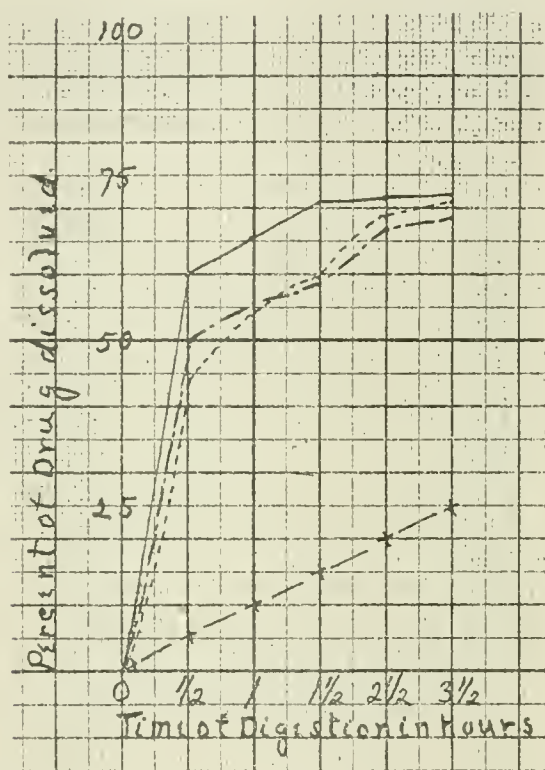


FIG. 2. TIME FACTOR IN SOLUTION OF TANNIN-PROTEIN COMPOUNDS

Digestion with bicarbonate-trypsin. — = protan; = tannin albuminate, Merck; -.-.- = tannalbin; +-+-+ = gallogen.

Compounds that have different solution should not be classed as identical with tannalbin. Protan should have somewhat different standards.

Egg-albumen precipitation by tannin albuminate

The percentage of the albumen precipitated by 2 per cent solutions of the astringents was as follows:

	HCl N/15	SODIUM BICARBONATE 1 PER CENT
Tannalbin, Knoll (Spec. 2).....	26	76
Albutannin, Merck (Spec. 24).....	26	39(?)
Albutannin, Calco.....	35	97
Tannic acid.....	63	100

It is seen that the compounds precipitate albumen both in acid and alkaline media, although not quite as much as tannin. Evidently, the tannin albuminates are actually capable of precipitating further quantities of albumen, under suitable conditions. The precipitation is more effective in the alkaline reaction.

The relative precipitant efficiency of the different compounds is proportional to their own solubility, as judged by the amount of nitrogen dissolved in the media, in the absence of added proteins. This was as follows, for the filtrates from 2 per cent suspensions, in terms of milligrams of nitrogen per 100 mgm. of drug:

	HCl N/15	SODIUM BICARBONATE 1 PER CENT
Knoll.....	2.0	1.6
Merck.....	2.0	0(?)
Calco.....	2.3	3.8

Tannin-proteinates and serum

The digests were made with 2 or 2.5 per cent of the tannin preparations. The filtrate of these were diluted with equal volumes of the serum solutions. The results are shown in table 6.

It is very evident that the phenomena are quite different for protan and tannalbin.

Protan precipitates almost as much as uncombined tannin, except in the trypsin digest. In this case, the tannin of the protan was probably used up during the digestion in precipitating the protein of the trypsin. The caseinate therefore protects but little against the direct effects of tannin.

Tannalbin is only slightly astringent in any of the digests. The largest precipitation is given by the neutralized acid-pepsin digest; turbidity is produced by the neutralized acid digest, and by the alkaline bicarbonate and trypsin digests. No precipitate is given by the un-neutralized acid digests; or by the neutral saline digests.

TABLE 6

Serum precipitation by tannin protein compounds

MENSTRUUM	TANNALBIN, SPECIMEN 9, 2 PER CENT	TANNALBIN, SPECIMEN 2, 2.5 PER CENT	PROTAN (CASEINATE), SPECIMEN 5, 2 PER CENT
Neutral saline.....	No precipitate	No precipitate	Precipitate
Hydrochloric, acid.....	No precipitate		Precipitate
Hydrochloric, then neu- tralized.....	Slight tur- bidity		
Pepsin, acid.....	No precipitate		
Pepsin, acid, then neutral- ized.....	Precipitate		Heavy precipi- tate
Bicarbonate, alkaline.....	Turbidity		Precipitate
Trypsin, alkaline.....	Turbidity		
Trypsin, neutralized.....	No precipitate	No precipitate	Turbidity
Bile, alkaline, neutralized....	No precipitate		
Carbonate, alkaline, neu- tralized.....	Slight tur- bidity		

Antihemolytic efficiency of tannin-proteinates

The results are shown in table 7. "Laking" is equivalent to *absence* of astringency.

The results again show the radical difference between the two compounds:

Protan protects completely against laking under all conditions, i.e., its astringency is essentially equal to that of unbound tannin.

Tannalbin is much less active. However, efficient protection, i.e., astringency, is exerted by the neutralized acid digest, by the neutral saline digest, and to a slighter degree by the alkaline digests. This agrees with the serum-test, except that the hemolysis

method shows astringency even for neutral saline digests, but only for concentrations above 1 per cent.

Kobert found the agglutination efficiency of neutral solutions of tannalbin as one-twentieth of that of tannic acid.

TABLE 7
Tannin-proteinates on hemolysis

MENSTRUUA	TANNALBIN, SPECIMEN 2, 2 PER CENT	TANNALBIN, SPECIMEN 9, 2 PER CENT	PROTAN, SPECIMEN 5, 2 PER CENT
Neutral saline, 2 per cent suspension.....		Laked	
Neutral saline, filtrates, 2 and 1 per cent.....	No laking		No laking
Neutral saline, filtrates, 0.5 per cent.....	Doubtful		
Neutral saline filtrates, 0.25 per cent.....	Laked		
Acid, neutralized, 2 per cent.....	No laking		
Acid, neutralized, 0.5 per cent.....	Laked		No laking
Acid, pepsin, neutralized.....			No laking
Bicarbonate, alkaline, 2 per cent.....	Doubtful	Laked	No laking
Bicarbonate, alkaline, 0.5 per cent.....	Doubtful		No laking
Bicarbonate, neutralized.....			No laking
Alkaline trypsin, neutralized.....			No laking
Carbonate, neutralized, 2 per cent.....		No laking	

Astringent taste of solutions

Filtrates of 2.5 per cent digests of tannalbin have little or no astringent taste. The neutral saline digest was non-astringent; the neutralized acid-pepsin and bicarbonate-trypsin digests were doubtful.

Astringent taste of the powders

Tannalbin. All samples taste slightly astringent when chewed; more so than tannigen, and less than protan or tannoform.

Protan. All samples are distinctly astringent from the start, more so than any other masked tannin compounds except tannoform.

Tannalbin on lung elasticity

The extension of the lungs is diminished by the neutral saline and by the neutralized acid and neutralized trypsin bicarbonate digests, as follows:

Neutral saline: 2.5 per cent, specimen 2, about like 1 per cent tannin.

Neutral saline: 1 per cent, specimen A, less than 0.25 per cent tannin.

Neutralized acid: 2.5 per cent, specimen 2, about like 1 per cent tannin.

Neutralized trypsin-bicarbonate: 2.5 per cent, specimen 2, about like 0.25 per cent tannin.

Summary of tannin-protein compounds

These do not comply with the claimed solubility. In fact, they are slowly and incompletely dissolved at all physiologic reactions, especially in the presence of the digestive ferments. The specimens, however differ materially, especially in their behavior toward trypsin-bicarbonate; the American products being more soluble than the original.

Limited, and especially slow solubility would probably extend the astringent action to lower levels of the intestine than the more rapidly soluble products. The rapid and complete solubility of protan is therefore probably undesirable if the action is to extend beyond the duodenum.

The commercial tannin-albuminate is capable of precipitating further quantities of albumen, even at the extreme reactions of the stomach and intestine. The precipitation is proportional to the solubility of the compounds. Alkalinity interferes less with the precipitation than the full acidity of the gastric juice.

The various tests of astringency show *protan* solution to act essentially like free tannin. Its slower solubility would, however, mitigate its action on the stomach, and extend it somewhat into the intestines.

Tannin-albuminates are very mildly astringent, by all the tests. The largest precipitation is given by the neutralized acid-pepsin digests; then come the neutralized bicarb-trypsin digests; and then the neutral digests. Unneutralized acid digests are not precipitant.

The results indicate the possibility of mild astringency in the mouth, which is confirmed by the taste. Only slight astringency

would occur in the stomach until digestion advanced, and then it would be largely suspended by the strongly acid reaction. Considerable astringency would develop in the duodenum, when the acidity is reduced. Some astringency would continue into the lower intestines, following the cleavage of the undissolved proteinate.

These properties would all be therapeutically desirable.

TANNIC ACETIC ESTER

Acetic acid esters of tannin were introduced into therapeutics by Hans H. Meyer, 1894, under the name *tannigen*. The common name *Acetannin* was introduced into N. N. R. to cover all the commercial brands.

Claims. Although the discovery of tannic acetic esters is universally credited to Hans Meyer, then of Marburg, the official United States patent application of September, 1894 (no. 522, 731) claims the discovery of the substance and of its therapeutic qualities for one "Jacob Meyer, chemist and doctor of philosophy," then of Frankfort-on-the-Main; and the patent was granted to him and his representatives.

Tannigen and the various brands of Acetannin are said to be prepared by the methods described in this original patent; that is by heating tannin and acetic anhydrid in the presence of glacial acetic acid. Here, however, another discrepancy occurs. The original patent describes the product of this process as "a mixture of monoacetylated and diacetylated gallic anhydrides," that is of mono- and di-acetyl tannins. The commercial products, however, are sold under the direct or implied claim that they are a definite compound, di-acetyl tannin, $C_{14}H_8(CH_3CO)_2O_9$; and not as indefinite mixtures. The varying properties of different specimens indicate that the information of the original patent was correct; that is, that the commercial article is a mixture and not a definite chemical.

Tannigen was described by H. Meyer as practically insoluble in water and therefore tasteless. It is dissolved by alkaline media (even sodium borate). On boiling, or on prolonged standing of the alkaline solutions it is saponified into acetic and gallic acid;

whilst ammonia produces acetic and tannic acids. Fresh solutions in sodium phosphate act as astringents; they precipitate glue and albumen and inhibit the secretion of frog's skin. The precipitates are redissolved by stronger alkalies, even borate; and these prevent the astringent action on the frog's skin. Apparently, therefore, the solutions contain free tannin, and this is probably the ordinary primary cleavage product with all dilute alkalies. E. Rost, 1897, confirmed that in the intestines tannigen is changed into tannic acid, and not into gallic acid.

On administering it to animals, H. Meyer found the stools more solid. The feces contained tannin, even after small doses, so that the action would extend to the end of the intestinal tract. The urine did not contain gallic acid, so that the absorption must have been slight.

Tannigen was subjected to clinical trial by Friedr. Müller, 1894. He found it free from side effects, and efficient in chronic diarrheas of various origin, especially tubercular. That is, it generally diminished the number and increased the consistence of the stools. In acute diarrhea, it was difficult to draw conclusions because of the uncertainty of spontaneous cessation.

O. Vierordt, 1896, an advocate of tannalbin, was less enthusiastic about tannigen. He claimed that tannigen is somewhat astringent in the mouth, and therefore also in the stomach. Long continued use may disturb the appetite. Its intestinal action is often not sufficiently powerful.

The later trend of the literature seems to be generally favorable, although not very critical. It is fairly well reflected in the following quotation from the circular of the manufacturers:

Professor C. A. Ewald, Berlin: The results, according to my experience and that of Biedert, Escherich, Kunkel, Hock and others, are nearly without exception prompt, and the drug is certainly more reliable than rhatany, catechu, hematoxylon, and other astringents.—Therap. Wochensch.

The use of acetannin therefore depends on the fact that it is almost insoluble; but that it is hydrolyzed under certain conditions, with the liberation of tannin. The rapidity of hydrolysis

varies with the conditions, and this probably explains some of the contradictory results. The ester is soluble in dilute alkali, and this doubtless favors hydrolysis; but the conditions of solution and hydrolysis are distinct. This has not been sufficiently clearly realized in the literature of tannigen. It will be shown that dissolved but undissociated acetyl-tannin does not precipitate proteins.

Some hydrolysis appears to occur even in the powdered drug; for each of four specimens of tannigen had a well-marked acetic odor.

Solubility of acetannin

The advertising matter of tannigen claims that this is insoluble in dilute acids and cold water, but dissolves in dilute alkalies.

The solubility, as estimated by Leech, showed considerable difference from the claims, and between the individual specimens, as will be seen from table 8.

TABLE 8
Solubility of tannin acetic esters

	MEDIAN AND RANGE OF PER CENT OF THE COMPOUND DISSOLVED BY				
	Water	Acid	Acid-pepsin	Bicarbonate	Bicarbonate-trypsin
Tannigen, claimed.....	Insoluble	Insoluble	Insoluble	Soluble	Soluble
Tannigen, found..	8.5 (8.5-17.2)		6.1 (3.8-17.4)	75 (31-100)	66 (32-100)
Acetannal Calco..	6.8	6.2	5.5	91	87

All the specimens are somewhat soluble in water and equally so in dilute acid. This has probably no practical importance, except as a test of quality.

The apparent difference in the alkali-solubility is important. Only two of the four preparations are completely or nearly completely soluble. The other specimens, both of the original German manufacture, are evidently contaminated with large quantities of insoluble by-products, which are probably to be considered as inert ballast, from the therapeutic standpoint. It is desirable that such specimens should be excluded by appropriate solubility tests.

The solubility appears slightly lower in the trypsin solution; this is doubtless due to the precipitation of some of the protein of the pancreas preparation.

Hydrolysis of acetannin

Leech noted a further point that is probably of material therapeutic importance, namely, that the hydrolysis of the dissolved

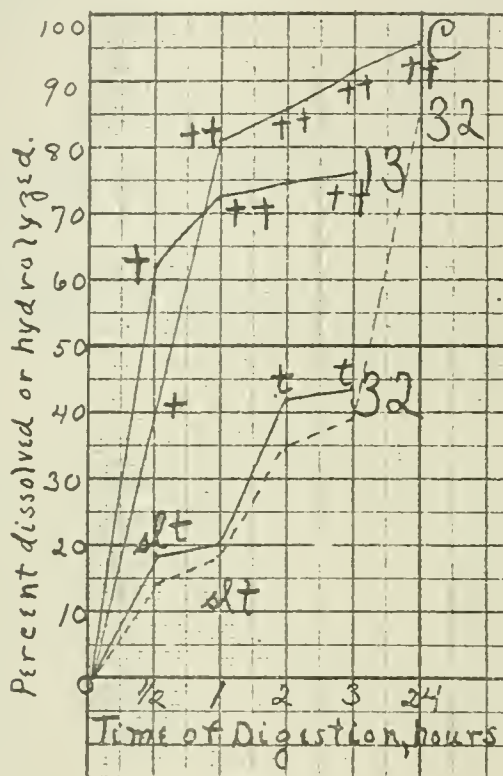


FIG. 3. TIME FACTOR IN HYDROLYSIS OF ACETANNINS

The specimens are digested with 1 per cent sodium bicarbonate at 40°C. Solution (----) is estimated by the weight of the undissolved residue. Hydrolysis (——) is determined by titration of the liberated acid. The precipitation of protein is shown by the symbols slt. = slight turbidity; t = turbidity; + = precipitate; ++ = heavy precipitate.

The following specimens were used: No. C., Acetannin Caleo; No. 13, Tannigen Bayer; No. 32, Tannigen Winthrop.

ester proceeds quite slowly, when digested with 1 per cent bicarbonate at 40°C. The presence of non-hydrolyzed esters is shown by precipitation on the addition of acid. The results for three specimens are shown in curves in figure 3. Theoretically 1 gram

of di-acetyl tannin, when completely hydrolyzed, should require 7.8 cc. of normal alkali. The astringency was estimated by the precipitation of egg-albumen solution.

The figure shows that the hydrolysis is only half completed in one-half hour, and reaches its acme in one or two hours. This slow liberation is doubtless a therapeutic advantage, in that it prolongs the action further into the intestines. The figure also shows that the precipitation of albumen is parallel to the hydrolysis; i.e., the ester as such is non astringent. That part which escapes hydrolysis is therefore inert ballast. The figure shows that this varies, in the different specimens at the end of three hours, from 10 to 60 per cent. Tests should be devised to limit this inert material to a minimum.

Egg-albumen precipitation by acetannin

The percentage of albumin precipitated by 2 per cent solutions was as follows:

	N/15	SODIUM BICARBONATE 1 PER CENT
Tannigen (Spec. 1).....	0	62
Acetannal.....	0	96

Acetannin does not precipitate in the acid medium, differing in this from the albutannins. This would be a therapeutic advantage. The precipitation in the alkaline medium is effective; the German specimen, however, being inferior to that of American manufacturers.

Serum precipitation by acetannins

The digests were made with 2 or 2.5 per cent of the tannin preparations. The filtrates of these were diluted with equal volumes of the serum solutions. The results are shown below:

Serum precipitation by acetannins

MENSTRUUA	TANNALBIN, SPECI- MEN 6, 2 PER CENT	TANNALBIN, SPECI- MEN 1, 2.5 PER CENT
Neutral saline.....	No precipitate	No precipitate
Hydrochloric, acid.....	No precipitate	
Hydrochloric, neutralized.....	Turbidity	
Pepsin, acid.....	No precipitate	
Pepsin, neutralized.....	Turbidity	
Bicarb, alkaline.....	Turbidity	
Trypsin, alkaline.....	No precipitate	
Bile, alkaline.....	Turbidity	
Carbonate, neutralized.....	No precipitate	

The acetannins again fail to precipitate at acid reaction. Turbidity, that is, slight astringent effects are given by the alkaline extracts, even at the alkaline reaction; and by the neutralized acid extracts. Neutral saline extract does not give turbidity.

These results agree essentially with those of the albutamines.

Antihemolytic efficiency of tannigen

The results are shown in table 9. "Laking" is equivalent to absence of astringency.

TABLE 9
Tannigen on hemolysis

MENSTRUUA	SPECIMEN 1 2 PER CENT	SPECIMEN 6 2 PER CENT
Neutral saline.....	Partly laked	Laked
Hydrochloric, neutralized.....	Not laked	
Bicarbonate, alkaline.....	Not laked	Laked
Trypsin, alkaline.....		Laked
Bicarbonate, neutralized.....	Not laked	
Carbonate, neutralized.....		Not laked

By this test, there appears considerable difference between the two specimens.

Specimen 6 was not astringent in neutral saline, or neutralized alkaline or trypsin digests; but only as the neutralized carbonate digest.

Specimen 1 gave complete protection for the alkaline digest, with or without neutralization; with the neutralized hydrochlorid digest; and partial protection with the saline digest.

The results illustrate the variable activity of the specimens. No. 6 would be very little astringent (it gave some turbidity with the serum test). Specimen 1 behaves essentially like the albutannins. Kobert found the agglutination efficiency of neutral solutions $\frac{1}{4}$ of tannin; a result that could only be anticipated with a decomposed product.

Astringent tests of tannigen solutions

Filtrates of 2.5 per cent saline and of neutralized pepsin-hydrochloric digests of tannigen had no astringent taste. The taste of neutralized trypsin-bicarbonate digest was doubtful. The astringent action in any case therefore cannot be strong.

Taste of acetannin in powder

The advertising claims that tannigen is tasteless.

This is not strictly true of any specimen, and far from true in some cases. Most specimens have an acid taste, which becomes somewhat astringent on chewing. The astringency is, however, very slight; tannopin being the only masked tannin compound, of those examined, that tastes less astringent than acetannin.

One specimen, however (German tannigen, no. 21) was very highly astringent; more so that any other specimen of any masked tannin compound. This again illustrates the unfortunate variability of the commercial drug.

Tannigen on lung-elasticity

The extension of the lungs is diminished by the neutral saline and by the neutralized pepsin hydrochloric digests; on the average about as much as by albutannin.

The results were as follows:

Neutral saline, 1 per cent specimen B: like 0.25 per cent tannin, more than tannalbin, less than tannoform.

Neutral saline, 2.5 per cent, specimen 1: Like 0.5 per cent tannin, less than tannalbin, more than tannoform.

Pepsin-hydrochloric, neutralized, 2.5 per cent, specimen no. 1: Like 2.5 per cent tannin; more than tannalbin or tannoform.

Trypsin-bicarbonate neutralized 2.5 per cent, specimen 1: Very little astringency. Evidently some technical error; perhaps combination with the protection of the trypsin.

Summary of tannic acetic esters

The commercial acetannin, and especially the original "tannigens" are variable mixtures. Some approach very closely to the ideal requirements; some consist mainly of insoluble and inert ballast, and some contain so much free tannin that they could not be classed as "masked" tannin compounds.

The best specimens are very slightly soluble in neutral and acid fluids, although they do have a slight astringent action even under these conditions. They dissolve in weakly alkaline fluids, but the solutions are not directly astringent. They become astringent as the ester is hydrolyzed, which occurs gradually and extends over two hours, under conditions approximating those of the intestines.

As compared with the tannin-proteinates, they would be somewhat less astringent in the stomach and upper intestines, and would develop their action gradually and progressively in the lower intestines. The astringency would always be mild.

Their special field, therefore, would appear to be when the astringent action is desired in the lower intestines. However, they do not deserve full confidence until the commercial products are more uniform. The high quality of some of the samples makes the outlook hopeful.

TANNOFORM

Composition and solubility. Tannoform is said to be a condensation product of tannic acid and formaldehyd, $\text{CH}_2(\text{C}_{14}\text{H}_9\text{O}_9)_2$. It is claimed to be insoluble in water or dilute acid, soluble in

alkalies, and is supposed to act in alkaline solutions both as tannin and as formaldehyd.

The actual solubility, as determined by Leech, on four specimens contrasts sharply with these claims:

	WATER	ACID	PEPSIN	ALKALI	TRYPSIN
Claimed.....	Insoluble	Insoluble	Insoluble	Soluble	Soluble
Found solution.....	32 (21-47)	37 (32-42)	15 (12-21)	87 (81-95)	84 (78-96)

The specimens of tannoform vary considerably in solubility. All, however, are fairly soluble in water and in dilute acid. The apparently lower solubility in the pepsin-acid indicates that the protein of the pepsin has been precipitated; i.e., that there is a slight astringent effect.

The solution in bicarbonate occurs almost immediately at 40°C. The residue that remains undissolved in bicarbonate does not yield formaldehyd on steam distillation, or to 10 per cent sodium hydroxid. This is evidently an impurity that would be useless therapeutically.

Of the part that goes into solution in bicarbonate, about one-third was still precipitated by acidulation, even after digestion with the bicarbonate at 40°C., for three and one-half hours. This part therefore has not been split into its components, as claimed.

The chemical data indicate that tannoform is not a single substance as the formula would imply, but that it is a mixture of unknown substances of prolematical behavior.

Egg-albumen precipitation by tannoform. No precipitate occurred in the N/15 acid solution; in the bicarbonate solution, 57 per cent of the egg-nitrogen was precipitated. Tannoform in the acid medium is therefore much less precipitant than the albuminate, in alkaline medium it is quite effectively precipitant, although not as active as the albuminates or acetannin.

Serum precipitation by tannoform. The digests were made with 2 or 2.5 per cent of the tannin preparations. The filtrate of these were diluted with equal volumes of the serum solutions. The results are shown in table 10.

The tannoform is non-precipitant in neutral digest, or in acid digests. After neutralization, the acid digest is feebly precipitant. The alkaline digests are feebly precipitant, before and after neutralization.

Antihemolytic efficiency of tannoform. The results are shown in table 11. "Laking" is equivalent to *absence* of astringency.

One specimen did not protect against laking in neutral, neutralized acid, or alkaline digests. The other specimen conferred

TABLE 10
Serum precipitation by tannoform

MENSTRUUA	2 PER CENT OF SPECIMEN 4	2.5 PER CENT OF SPECIMEN 3
Neutral saline.....	No precipitate	No precipitate
Hydrochloric acid.....	No precipitate	
Hydrochloric, then neutralized.....	Turbid	
Pepsin-acid.....	No precipitate	
Pepsin-acid, then neutralized.....	No precipitate	No precipitate
Bicarbonate, alkaline.....	Turbid	
Bicarbonate, alkaline, then neutralized...	Turbid	
Trypsin, alkaline.....	Turbid	
Bile, alkaline.....	Turbid	
Carbonate, then neutralized.....	Slight precipitate	

TABLE 11
Tannoform on hemolysis

MENSTRUUA	2 PER CENT OF SPECIMEN 4	2.5 PER CENT OF SPECIMEN 3
Neutral saline.....	Laked	Partly laked
Hydrochloric acid, then neutralized.....	Laked	
Bicarbonate, alkaline.....	Laked	No laking
Trypsin, alkaline.....	Laked	
Carbonate, neutralized.....		Laked

partial protection in the neutral digest, and complete protection in the bicarbonate digest.

Kobert found the agglutinative efficiency half as great as with tannic acid. This conclusion seems incredible.

Astringent taste of tannoform solutions. The filtrate of the 2.5 per cent digest tasted slightly astringent. The neutralized acid-pepsin digest was not astringent; the neutralized trypsin bicarbonate digest was doubtful.

Astringent taste of tannoform powder. All specimens are quite astringent from the start; more so than any of the other masked tannin compounds.

Tannoform on lung-elasticity. The extension of the lung is markedly diminished by the saline and still more by the neutralized acid-pepsin digest, as follows:

Neutral saline, 2.5 per cent, specimens C and 3: Marked astringent effect, somewhat more than other masked compounds, and about equal to 0.5 or 1 per cent tannin. Lungs appear hardened.

Neutralized pepsin-acid: Even more astringent than the saline digest.

Neutralized bicarbonate: The extension seems to be but little altered; but this is probably a technical error (leakage?), for the lung appears semi-leathery.

Summary of tannoform. This is an impure product, with variable composition. It is somewhat soluble in water and more so at the gastric acidity. Its astringent characters resemble those of albutannin rather than acetannin; i.e. it would act in the stomach more than does acetannin. There are no data as to the liberation of formaldehyd, but free formaldehyd would be therapeutically undesirable.

It is difficult to conceive any advantage of tannoform over the albutannin or acetannin. It is therefore superfluous; and in view of the possible formaldehyd liberation, it appears theoretically undesirable.

TANNOPIN

This is said to be a definite condensation product of tannic acid and hexamethylenamin.

Solubility of tannopin. Tannopin is also claimed to be insoluble in water and dilute acids, but to be decomposed by dilute alkalies and then to act as tannin.

Leech's examination of a specimen shows the usual discrepancy between the claims and the findings:

	WATER	ACID	PEPSIN	ALKALI	TRYPSIN
Claimed.....	Insoluble	Insoluble	Insoluble	Soluble	Soluble
Found solution.....	17	32	20	26	24

The claimed contrast of solubility in alkalies as against water and acid does not exist. The solubility characters speak against therapeutic efficiency; although it is conceivable that the action in the lower intestines might be more efficient than with more soluble compounds.

Egg-albumen precipitation by tannopin. None was precipitated in the acid medium; 50 per cent were precipitated in the

TABLE 12
Tannopin on serum precipitation and hemolysis

MENSTRUUM	SERUM	BLOOD
Neutral saline.....	No precipitate	One laked, another partly laked
Hydrochloric, acid.....	No precipitate	Doubtful
Hydrochloric, neutralized.....	No precipitate	
Pepsin, acid.....	No precipitate	
Pepsin, neutralized.....	Turbidity	One laked, another not laked
Bicarbonate, alkaline.....	No precipitate	
Trypsin, alkaline.....	No precipitate	Laked
Trypsin, neutralized.....	Turbidity	Not laked
Carbonate, neutralized.....	Slight precipitate	

bicarbonate medium. The bicarbonate precipitation is about the same as with tannoform; and smaller than with albutannin or acetannin.

Tannopin on serum-precipitation and hemolysis. The digests were made with 2 per cent of specimen 12. The filtrates of these were diluted with equal volumes of the serum solutions. The results are shown in table 12. With the blood experiments, laking means that the solution is *not astringent*.

Astringent taste of tannopin solutions. Filtrate of the 2.5 per cent digests have little or no astringent taste. The neutral saline and the neutralized pepsin hydrochloric digests were non-astringent. The neutralized trypsin-bicarbonate digest was doubtful.

Astringent taste of the powder. Two specimens were entirely non-astringent.

Lung-elasticity. This was not tested.

Summary of tannopin. This is only very feebly astringent; about equally so in neutralized acid and neutralized alkaline digest. It is apparently less effective than albutannin or acetanin, and is at least superfluous.

TANNISMUTH

Composition and solubility. Tannismuth is said to be bitannate of Bismuth, approximately $\text{Bi}(\text{OH})(\text{C}_{14}\text{H}_9\text{O}_9)_2$. It is claimed as insoluble in water, but soluble in dilute acids. It is also claimed that one molecule of tannin is split off at once on ingestion; but that the other tannin molecule is split off more slowly, so as to act in the intestine. It is employed against diarrhea, to produce "the combined effect of bismuth and tannic acid."

Leech found a specimen to behave as follows:

	WATER	ACID	PEPSIN	ALKALI	TRYPSIN
Per cent soluble.....	18.2	25	14.8	14.6	12.8

These findings indicate that a part dissolves in water and somewhat more in dilute acid. The pepsin experiment shows that this can precipitate protein and is presumably tannin. There is, however, no indication that any further tannin is split off by bicarbonate. In fact, the solubility in alkali is even less than in water. The preparation would act essentially after the manner of insoluble bismuth salts, plus a slight tannin effect that would probably be expended entirely on the stomach.

Tannismuth on serum precipitation and hemolysis. The digests were made with 2 per cent of specimen 10. The filtrates were diluted with equal volumes of serum solution. The results are shown in table 13. In the blood experiments, laking means that the solution is *not* astringent.

Taste of tannismuth powder. This is distinctly astringent from the start, ranging between tannalbin and protan.

Summary of tannismuth. The tannin of this preparation seems to be equally soluble in acid and alkaline media; its astringent action would probably be expended in the stomach, so that it would act in the intestines probably mainly as an insoluble bismuth salt.

TABLE 13
Tannismuth on serum precipitation and hemolysis

MENSTRUUM	SERUM	BLOOD
Neutral saline.....	No precipitate	One laked, another not laked
Hydrochloric, neutralized.....	Slight precipitate	One partly laked another not laked
Pepsin acid, neutralized.....	Precipitate	Not laked
Bicarbonate, alkaline.....		Not laked
Bicarbonate, neutralized.....	Slight precipitate	Laked
Trypsin, neutralized.....	Precipitate	Not laked

GALLOGEN

Comparison and solubility. "Gallogen" is described as anhydrous ellagic acid. It is claimed as insoluble in water and dilute acids, and as soluble to 2 per cent in alkaline media. It is thus supposed to dissolve slowly in the intestines and to act there as astringent. The solutions oxidize readily.

The specimen examined by Leech behaved as follows:

	WATER	ACID	PEPSIN	ALKALI	TRYPSIN
Claimed.....	Insoluble	Insoluble	Insoluble	Sparingly soluble	Sparingly soluble
Per cent found soluble....	18		15.9	14.9	17.2

The data show that the compound is sparingly and about equally soluble in all the media.

Egg-albumen precipitation by gallogen. None was precipitated in N/15 acid; in bicarbonate, 58 per cent of the albumen was precipitated.

Gallogen on serum precipitation and hemolysis. The digests were made with 2 per cent of specimen 11. The filtrates were

diluted with equal volumes of serum solution. The results are shown in table 14. In the blood experiments, laking means that the solution is *not* astringent.

Taste of the powder. This is at first slightly salty and acid, then slightly astringent, ranging between tannigen and tannalbin.

Summary of gallogen. This is feebly astringent, rather more so with alkaline than with acid digests (after neutralization). The chemical data are not sufficient to define its probable usefulness.

TABLE 14

Gallogen on serum precipitation and hemolysis

MENSTRUUA	SERUM	BLOOD
Neutral saline.....	No precipitate	One laked, another not laked
Hydrochloric, neutralized.....	No precipitate	One laked, another not laked
Pepsin acid, neutralized.....	No precipitate	Laked
Bicarbonate, alkaline.....	No precipitate	Not laked
Bicarbonate, neutralized.....	No precipitate	Not laked
Trypsin, neutralized.....	No precipitate	Laked

THE INFLUENCE OF PEPSIN IN ACID DIGESTS ON THE ASTRINGENCY OF MASKED TANNINS

It is generally stated that peptic digestion diminishes the precipitation of proteins by tannin. This is probably an error based on neglect of the influence of the reaction in digestion experiments; for as was shown in the first paper of this series, peptones and native proteins behave essentially alike toward tannin.

There is another manner however, in which pepsin might affect the astringency of masked tannin compounds, and that is by increasing their solubility. This would be expected with the heat-coagulated protein of tannalbin and with the acid-insoluble casein of protan.

The results are shown in table 15.

TABLE 15
Comparison of HCl and pepsin HCl

	PER CENT DISSOLVED IN		SERUM PRECIPITATION (AFTER NEUTRALIZATION) BY	
	HCl	Pepsin HCl	HCl	Pepsin HCl
Protan.....	37	79	+	++
Tannalbin.....	3	76	t slight	+
Tannopin.....	20	26	0	t
Tannigen.....	5.8	6.2	t	t
Tannismuth.....	25	15	+	+
Gallogen.....	20	16	0	0
Tannoform.....	37	15	t	0

THE INFLUENCE OF TRYPSIN IN BICARBONATE DIGESTS ON THE
ASTRINGENCY OF MASKED TANNINS

Trypsin might conceivably influence the solubility and therefore the activity of these compounds; in the case of the albuminates by dissolving coagulated albumen; in the case of esters, by promoting their saponification.

The results of Leech (table 16) showed that trypsin does actually increase the solubility of tannalbin; but that it has no effect on any of the other masked compounds.

TABLE 16
Influence of trypsin on solubility in bicarbonate (from Leech)

	PER CENT DISSOLVED IN	
	Bicarbonate	Bicarbonate and trypsin
Tannalbin.....	45	60
Protan.....	90	87
Tannigen.....	82	76
Tannoform.....	94	93
Tannopin.....	24	24
Gallogen.....	15	17

The precipitation and hemolysis experiments indicate that the trypsin-bicarbonate digests are somewhat less astringent than the simple bicarbonate digests; but this doubtless is deceptive; i.e., precipitation of the trypsin-protein has already bound a part of the tannin.

THE INFLUENCE OF BILE-SALTS ON THE ASTRINGENCY OF MASKED TANNIN

Since bile salts occur in the intestines and increase the solubility of some substances, they were tried in some of the precipitation experiments with tannalbin, tannigen and tannoform. The results were negative, i.e., identical with those of bicarbonate in the absence of bile salts.

SUMMARY AND CONCLUSIONS

The astringencies of "masked tannin compounds" were compared with each other, and with those of tannic acid, under conditions simulating those occurring in the digestive tract. Various criteria of astringency were applied, including a new method employing blood-corpuscles. All methods gave fairly concordant results. The simplest, and therefore the most satisfactory appeared to be the precipitation of protein-solutions. This, in relation to the solubility and taste of the drug, should give a fairly complete picture of its field of usefulness.

Great variations in the composition and properties of different specimens of the commercial products render their exact classification difficult. However, the following conclusions appear justified:

Tannin-protein compounds. Contrary to prevailing opinions, these dissolve rather better in artificial acid-gastric juice than in bicarbonate-trypsin solutions. Their solubility is so slow, however, that they could be only slightly astringent in the stomach. Considerable astringency would develop in the duodenum, when the acidity is reduced; and the effects would continue somewhat into the lower intestine, following the cleavage of the undissolved proteinate. Slow solubility in bicarbonate solution is therefore a desideratum if the action is intended to continue beyond the duodenum.

Tannin-acetyl esters. Commercial brands are evidently mixtures of varying quality; some specimens apparently containing considerable free tannin. The best specimens, however, appear fairly uniform, and but slightly soluble and astringent in acid

solution. Bicarbonate dissolves them, and hydrolyzes them slowly. The astringency goes parallel to the hydrolysis, so that the action would continue for several hours, and could thus extend into the lower intestines. However, they do not deserve full confidence until the commercial products are more uniform.

Other compounds. The properties of those that were investigated do not appear promising.

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MAGNESIUM SULPHATE IN ARSENIC POISONING

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Arsenic poisoning from salvarsan administration is an occasional accident against which the doctor has no direct weapon. In connection with an attempt to bring about myocardial poisoning in animals by arsenic, my attention was called to Sieber's (1) work on the prolongation of life in rabbits by giving magnesium sulphate subcutaneously after a lethal dose of arsenic. His conclusions seemed to have a direct bearing on the salvarsan problem, and at the same time offered an opportunity to study the effect of the drug on the heart muscle. The series of experiments reported below were therefore undertaken at the suggestion of Dr. A. D. Hirschfelder.

With Sieber's figures as a basis, ten rabbits were used in establishing the correctness of his dosage. Fowler's solution was used hypodermically and by mouth in varying amounts, and it was found that death occurred, as he had reported, after administration of 7 mgm. per kilogram of arsenic (0.7 cc. Fowler's solution) subcutaneously or after twice that amount (0.14 cc.) by stomach tube. Death occurred usually within forty-eight hours, and autopsy showed in some cases no gross pathological lesion, in others hemorrhagic areas in the kidney and evidences of congestion of the intestinal vessels.

With Sieber's work on the lethal dose apparently corroborated on this small series, the next step was to test the effect of magnesium sulphate solution. In the work done by Meltzer (2) on tetanus, it was given to produce anesthesia and it seemed advis-

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able to administer anesthetic doses of the salt before giving the poison. If the magnesium sulphate counteracts the poisoning effect of the arsenic by forming an insoluble compound in the tissues as Sieber suggests then the larger the dose that could be given safely the better the chance of getting the antidotal effect. Consequently a series of twelve animals were given magnesium sulphate before the Fowler's solution with the methods described.

Rabbits were weighed and given the dose of magnesium sulphate in 25 per cent solution under the skin of the back. Then the dose of Fowler's solution (0.7 mgm. As_2O_3 per kilogram) was given hypodermically twenty to thirty minutes later before full anesthetic effect had been obtained. Each group of rabbits receiving magnesium sulphate and arsenic was controlled by an equal group receiving arsenic alone. Three rabbits were given anesthetic doses of the salt (1 to 1.5 grams per kilogram) followed by Fowler's solution. The first one died in twelve hours after both magnesium sulphate and arsenic. All six were dead in three days and those "protected" by anesthetic doses of the sulphate averaged a distinctly shorter duration of life than the other three.

In another group of six, the magnesium sulphate rabbits died earlier in general than those without it. One animal lived three weeks and it was found to have received a dose too small for anesthesia (0.5 gram per kilogram, Sieber's maximum dose of safety). To summarize this group of twelve, the five rabbits with anesthetic doses of the magnesium sulphate averaged shorter lives than the six who had received arsenic alone. The one receiving a non-anesthetic dose of the salt lived much longer than any of the others. The magnesium sulphate in an anesthetic dose did not prolong the life of the arsenic-poisoned rabbit, but accelerated his death. The smaller dose in one case prolonged life distinctly.

To establish the positive action of magnesium sulphate against the toxicity of arsenic and to determine the minimum amount giving this action was the purpose of the next series of experiments.

The same dose of arsenic (7 mgm. per kilogram) was used in each case except in a short series in which 8 and 10 mgm. were

TABLE 1

Showing dosage of arsenic and magnesium sulphate and length of life in fifty rabbits

RABBIT NUMBER	HOURS OF LIFE	DOSE OF ARSENIC	DOSE OF MAG- NESIUM SULPHATE	REMARKS
		<i>mgm.</i>	<i>grams</i>	
1	528	7	0.5	Liver at autopsy—moderate hyperemia and fatty degeneration
2	528	7	0.5	Kidney showed low grade nephrosis. Liver slight fatty change
3	912	7	0.5	Emaciated. No autopsy done
4	60	7	0.5	Marked necrosis of kidney and liver. Fatty degeneration of kidney, liver and heart
5	18	7	1.5	Necrosis of kidney tubules
6	18	7	1.0	Congestion of kidney with mild nephrosis. Hemorrhages in heart muscle
7	18	7	1.5	Cloudy swelling of kidney tubules
8	24	7	1.0	No gross pathologic lesions
9	42	7	None	Fatty granules in kidney, heart, liver and lung. Bronchopneumonia
10	30	7	None	Hemorrhages in kidney and liver. Some fatty degeneration
11	276	7	None	Some fatty granules in kidney and liver
13	288	7	0.25	Necrosis of liver cells
14	288	7	0.25	Congestion of liver and kidney glomeruli
15	12	7	None	
16	48	7	None	
17	40	7	None	
18	210	7	None	
19	2064	7	0.5	
20	2304	7	0.5	Emaciation. White firm nodules in liver. No microscopic study
21	1752	7	0.25	Organs appear normal grossly
22	1704	7	0.25	Emaciation. White nodules in liver
25	264	7	None	Liver appears pale in gross specimen. No microscopic study
26	384	7	0.20	Right ventricle dilated
28	36	7	0.20	Increased fluid in pleural cavities. Broncho-pneumonia.
29	120	10	None	Diarrhea before death
30	48	10	None	Diarrhea
31	48	10	None	Diarrhea
32	48	10	0.25	Increased fluid in pericardium
33	48	10	0.25	No gross pathologic lesions
34	24	10	0.25	Consolidation of portions of lungs
35	1272	8	None	
36	24	8	None	

TABLE 1—*Continued*

RABBIT NUMBER	HOURS OF LIFE	DOSE OF ARSENIC	DOSE OF MAG- NESIUM SULPHATE	REMARKS
		<i>mgm.</i>	<i>grams</i>	
37	384	8	None	
38	24	8	0.5	No autopsy
39	48	8	0.5	No autopsy
40	360	8	0.5	No autopsy
41	312	8	None	
42	24	8		
43	72	8	0.5	Liver pale and yellowish. Kidney pale with hemorrhagic areas
44	72	8	0.5	
45	60	8	0.25	
46	24	8	0.25	
47	168	7	None	
48	168	7	None	
49	112	7	1.0	Three doses magnesium sulphate, 24 hours apart
50	168	7	1.0	Three doses magnesium sulphate, 24 hours apart
51	696	7	None	
52	364	7	None	
53	48	7	None	
54	48	7	0.75	Magnesium sulphate in 2 doses a day between

used as will be recorded later. The dose of magnesium sulphate varied from 0.20 gram to 1.5 grams in single doses, and in several cases the doses were repeated. The arsenic was given subcutaneously in the back in the form of Fowler's solution, and the sulphate in a 25 per cent solution was injected from one to five minutes later. Autopsies were performed on many and microscopic study of the organs made.

A series of fifty rabbits receiving arsenic in toxic doses is here reported, and of these twenty-nine received magnesium sulphate in antidotal amounts. The twenty-one rabbits receiving arsenic alone averaged 219 hours (9 + days) of life. Of these thirteen received 7 mgm. per kilogram (Sieber's lethal dose) and lived 182 hours, five received 8 mgm. and lived 403 hours (16 + days) each, and three received 10 mgm. and lived 72 hours each on the average, as recorded in tables 1 and 2.

The magnesium sulphate rabbits, twenty-nine in number, lived 415 hours (17 + days) on the average. They may be grouped by the amount of arsenic used and by the dosage of the salt given to counteract it. Nineteen of the animals received 7 mgm. of As_2O_3 per kilogram and were given magnesium sulphate from 0.20 to 1.5 grams per kilogram as tabulated in table 3.

TABLE 2

Showing the average length of life after administration of arsenic

DOSE OF As_2O_3	NUMBER OF ANIMALS	TOTAL HOURS	AVERAGE HOURS
<i>mgm. per kgm.</i>			
7	13	2366	182
8	5	2016	403
10	3	216	72
	21	4598	219

TABLE 3

DOSE OF As_2O_3	MAGNESIUM SULPHATE PER KILOGRAM	NUMBER OF ANIMALS	HOURS	AVERAGE
<i>mgm. per kgm.</i>				
7	1.5	2	36	218
7	1.0 (3 doses)	2	280	140
7	1.0 (1 dose)	2	306	153
7	0.75 (2 doses)	1	48	48
7	0.50	6	6396	1066
7	0.25	4	4032	1008
7	0.20	2	420	210
8	0.50	5	576	115
8	0.25	2	84	42
10	0.25	3	120	40
		29	12,034	415

The average length of life was longer after magnesium sulphate was given than after arsenic alone.

Those receiving the minimum lethal dose of arsenic with the moderate dose of magnesium sulphate (0.25 to 0.50 gram per kilogram) had the longest average lives (1000 or more hours). Those receiving moderate doses of arsenic with large doses of the sulphate (1.5 grams) and those with large doses of arsenic and

moderate doses of the sulphate lived the shortest lives. However, there is a marked variation shown in susceptibility to the drugs and the three animals receiving 10 mgm. of the arsenic with 0.25 gram of the sulphate, the two receiving 8 mgm. arsenic with 0.25 gram sulphate and the one receiving 0.7 mgm. arsenic with a 0.75 gram sulphate in divided doses lived between 40 and 48 hours. It will also be noticed that in the arsenic controls those receiving 8 mgm. of the poison lived an average of 403 hours, while those with 7 mgm. averaged only 182 hours of life. Gross differences like these with methods and conditions of administration unchanged would, it seems, mean individual variations in susceptibility.

Comparing the length of life of the animals receiving arsenic alone and those receiving the same amount but combined with magnesium sulphate, it is found that those with a minimum lethal dose (7 mgm.) averaged much longer lives after magnesium sulphate than those without it—695 hours as compared with 182 hours—while those with the larger dose of arsenic lived longer without the sulphate than with it.

In giving arsenic preparations clinically, a very wide difference in tolerance to the drug is observed in different individuals. A few days of small doses of Fowler's solution may bring about early symptoms of poisoning in some patients while others may take many times the quantity. So also it was found in rabbits that the lethal dose varied markedly. The earliest death took place in 12 hours. The longest life was 2304 hours (3 months).

Roth (3), in connection with work on the various salvarsan preparations found this susceptibility so great that he considered the lethal dose to be the dose which killed 75 per cent of the animals in two weeks. He did not call death due to arsenic poisoning if the animals lived over two weeks. He discharged the animal if it was still alive at the end of thirty days. His reason for choosing this time limit was that Burnashoff (4) (1912) found only traces of arsenic in the various organs of rabbits three weeks after administration of salvarsan intravenously.

With Roth's figures in mind the lethal dose of arsenious acid would be less than 7 mgm. if given alone and much more than

7 mgm. if given with magnesium sulphate. Of thirteen rabbits poisoned with 7 mgm. per kilogram eleven or 84.6 per cent died in less than two weeks. Of nineteen rabbits receiving both

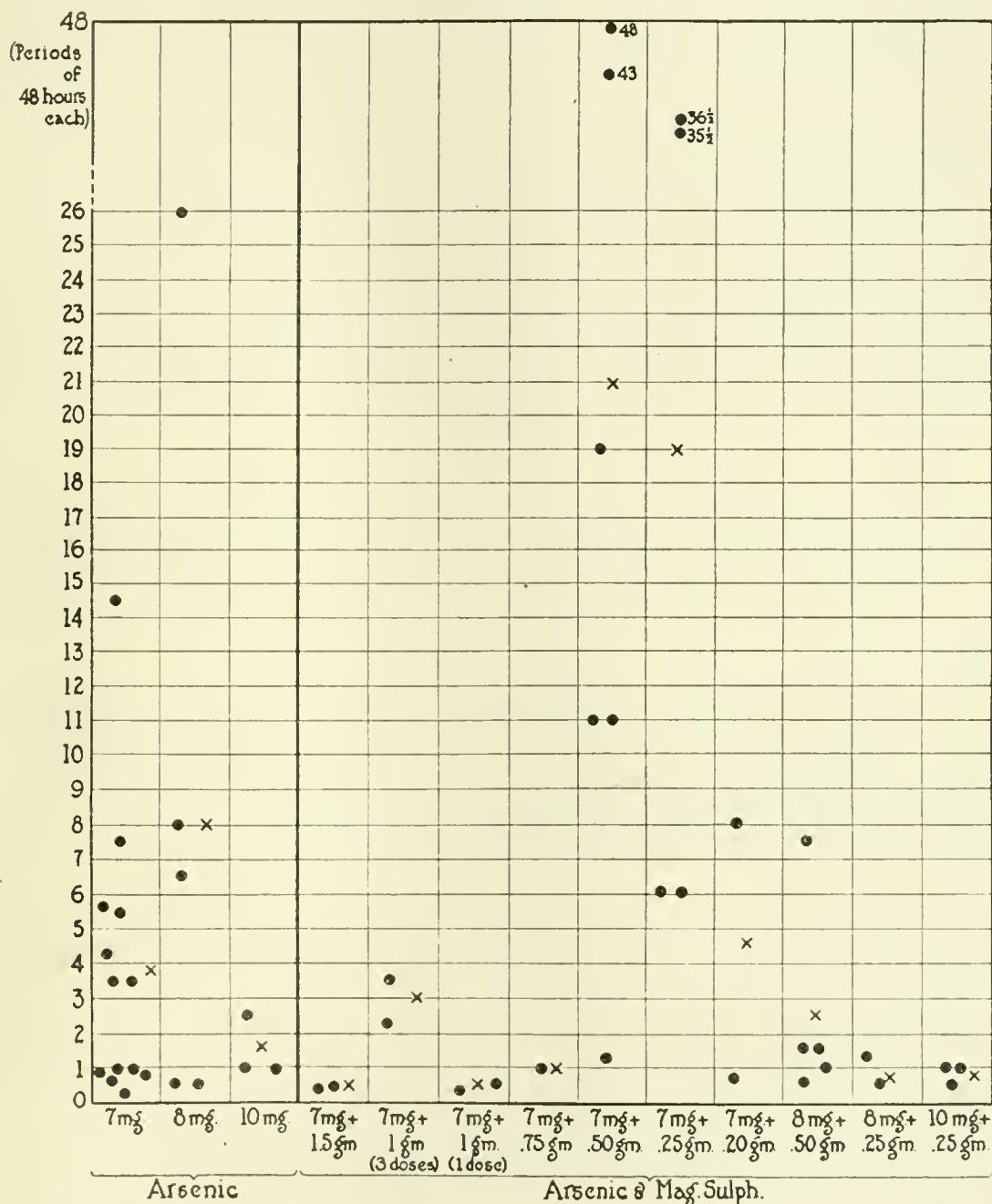


FIG. 1. LENGTH OF LIFE (IN 48 HOUR PERIODS) OF RABBITS GIVEN POTASSIUM ARSENITE ALONE, AND OF ANIMALS GIVEN POTASSIUM ARSENITE AND LATER MAGNESIUM SULPHATE

Doses shown represent milligrams per kilogram of As_2O_3 and of milligrams of So_4 respectively. The black dots represent the length of life of individual rabbits, the crosses represent the averages.

Fowler's solution and magnesium sulphate only eleven or 57.8 per cent died in less than two weeks. Contradictory to these findings, however, were the results in a smaller series in which 8 mgm. of arsenic seemed too small for a lethal dose causing death of only 60 per cent while if combined with the sulphate death occurred before two weeks in 85.7 per cent, as shown in table 4. 80.9 per cent of the arsenic poisoned animals, and 68.9 per cent of those receiving magnesium sulphate died within two weeks.

Autopsies were performed on a large number of the series and microscopic sections made in many cases.² Some cases showed no pathological lesions; some showed evidence of congestion of vessels of the intestinal walls; some prolonged cases showed emaciation; in some cases kidneys showed hemorrhagic areas.

TABLE 4

DOSE	ARSENIC				MAGNESIUM SULPHATE				
	Number	Average hours	Number died less than 2 weeks	Per cent died less than 2 weeks	Number	Total	Average hours	Number died less than 2 weeks	Per cent died less than 2 weeks
<i>mgm.</i>									
7	13	182	11	84.6	19	11,518	606	11	57.8
8	5	403	3	60.0	7	660	94	6	85.7
10	3	72	3	100.0	3	120	40	3	100.0

80.9 per cent of the arsenic poisoned animals as opposed to 68.9 per cent of those receiving magnesium sulphate died within two weeks.

A few animals were kept in metabolism cages and a study of the urine made for albumin and casts, but none so observed showed any signs of urinary abnormality.

Although the evidence points towards a prolongation of life by magnesium sulphate, the antidote is so toxic in itself and the poison so variable in its effect that it is impossible to say conclusively whether death in a given case is due to poison or to antidote. Further work must be done to find a compound of magnesium less toxic than the sulphate, which will have the antagonistic effect toward arsenic without the danger to the

² Examination of pathological specimens done by Dr. Theodore Sweetser of the Department of Pathology, University of Minnesota.

animal. Investigations are now being carried out with magnesium benzoate, with some promises of good results, but not enough material has been gathered for a report. The time of giving the magnesium in relation to the administration of the arsenic may be a factor to consider, though so far whether it was given one-half hour before or a few minutes later has apparently made no difference in results.

Definite conclusions can not be drawn in regard to the action of magnesium sulphate in arsenic poisoning, but results may be summarized as follows:

1. Magnesium sulphate has prolonged the average life of a series of fifty rabbits poisoned by arsenic from 219 hours to 415 hours on the average, but cannot be said to have saved life in rabbits.

2. Magnesium sulphate is toxic in large doses and to some extent in medium sized doses.

3. There is a marked variation of individual susceptibility to arsenic poisoning.

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SOME OBSERVATIONS UPON THE BEHAVIOR OF A FIXED OIL (PEANUT OIL) INJECTED INTRAPERITONEALLY

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This study was made incidentally to the investigation of another problem in which fixed oil was injected into the peritoneal cavity as a solvent for fat soluble material. Although it has long been known that when Linamentum Camphorae, U. S. P., a preparation of cottonseed oil and camphor intended for external use only, is injected into the subcutaneous tissue it may remain there for a long time and is prone to lead to pathological processes (1), very few data concerning the behavior of fixed oil within the peritoneal cavity exist. Corper (2) injected into the peritoneal cavity of guinea-pigs solutions of Sudan III in oil or butter, and upon performing post mortem examination observed the following: The presence of much of the injected oil free within the peritoneal cavity; a superficial staining of the abdominal fat, with the exception of that at the head of the testes: fibrin-like masses around the liver and under the diaphragm; and stained oil in some of the lymph glands.

In the experiments herein reported, from 1 to 10 cc. of cold pressed peanut oil was injected into the peritoneal cavity of rats mice, guinea-pigs, rabbits and cats, under clean but not aseptic technique. A few animals received oil containing 1 per cent lecithin, and a few more received oil containing Sudan III (C. P. melting point 200°C. uncorrected).¹ The animals were well fed and at no time was the effect of inanition upon the absorption

¹ This material was prepared in the Color Laboratory of the Bureau of Chemistry.

of the oil tested. After their general behavior was noted for varying lengths of time the animals were killed and post mortem examination performed. The amount of oil remaining within the peritoneal cavity, the pathological effect of the oil upon the peritoneum, the intensity of staining of different parts of the peritoneum and the disappearance of dye both from the oil and from the stained parts were determined by inspection. In two instances the percentage of dye in the free oil remaining in the peritoneal cavity was determined in a Dubosque colorimeter.

As far as was observed no untoward physiological effect was produced in any of the animals by the oil. The rats grew well, pregnancy occurred and a few litters were raised. There was never any oil or dye noted in the region of the vagina. Rabbits and guinea-pigs remained in good health, a point which is particularly interesting in view of the reputed harmfulness for these animals of a diet containing unsaturated fatty acid esters. Mice and cats seemed to be unharmed by the oil.

RESULTS FROM POST MORTEM EXAMINATION OF ANIMALS

Rats. Two and three months after the injection, a considerable amount of the oil which had been injected usually was found free within the peritoneal cavity. The use of 1 per cent lecithin in the oil did not seem to favor materially its rate of disappearance from the peritoneal cavity. Each of eight rats, which received 8 cc. of oil containing 0.2 per cent Sudan III, were examined after a period of twenty-four weeks, in one no oil was found, in another only a film of oil covered the peritoneum, while in the remaining six varying amounts of free oil were present. In several of these rats chains of oil globules were found retroperitoneally, running in the general direction of the lumbar nerves.

The superficial character of the staining of the fatty deposits, in the rats which received the oil containing the dye, was very apparent in cross section of the thicker parts. Although the staining was relatively greater the more concentrated the dye, the most noticeable feature was the different degree or intensity of staining of various parts. The omentum, broad ligament and

mesentery were the most deeply colored. The margin of the mass of fat attached to the testicle was next in order, while the base or pedicle of the latter, together with the lumbar fat, was the least stained. The peritoneum over the abdominal muscles also appeared slightly stained. Although no observations were made as to the rate at which the stained oil or dye was first taken up, a fading of the intensity of the staining of the peritoneum was plainly noticeable at the beginning of the third month. The samples of free oil from two rats which were killed at the end of the twenty-fourth week were found to have suffered a loss in dye, as determined in the colorimeter, of 85 and 95 per cent, respectively.

The least noticeable feature to the inexperienced eye, although a very important one, was the occurrence of single or flattened conglomerations of cyst-like spheres containing oil. The individual spheres varied in size, the largest being several millimeters in diameter. They usually became very evident by the end of the first month and were found loosely attached to the peritoneum, chiefly on margins of the liver and the dome of the diaphragm. Occasionally a single cyst attached by a long thread-like ligament was seen. At first the walls of these cyst-like bodies were very thin and transparent, but later became thicker and opaque. Their number and distribution, which varied in different individuals, were brought out very clearly by the use of the stained oil. By the twenty-fourth week they contained much semi-solid matter, while a few were distinctly gritty, suggesting a calcification process. At this stage it was still possible to express from these bodies the stained oil which appeared quite deeper in color than that found free in the peritoneal cavity. Minor histological study of these cyst-like bodies was made and the presence of a connective tissue capsule, capillaries and granular contents ascertained. The nature of their lining, however, was not determined.

Mice. These animals were injected only with the unstained oil. After one month a considerable amount of the injected oil remained free in the peritoneal cavity. The cyst-like bodies referred to were also noted.

Cats. A few cats were injected with oil or oil containing Sudan III. In contrast to the behavior of the rats and mice, the cats showed a tendency for a little serous exudation into the peritoneal cavity, in which the oil sometimes formed small droplets. In only one cat were a few cyst-like bodies found. In another cat, the injected oil was traced by means of the dye, from the base of the uterus up the lumbar lymphatics. In one cat, the oil found at the end of the 6th month had a distinctly rancid odor.

Rabbits and guinea-pigs. In these animals light yellow fibrin-like shaggy and cheesy material formed within twenty-four to forty-eight hours. There was also a small amount of serous exudate. These phenomena did not disappear by the beginning of the second month, and oil droplets were still present within the cheesy material, as well as in the serous exudate.

DISCUSSION AND CONCLUSIONS

Although the purpose of this investigation was accomplished in the demonstration of the apparent harmlessness and the relatively slow absorption of fixed oil injected into the peritoneal cavity, a more detailed study of the problem would seem to offer a very interesting field of investigation. The different intensity of staining of different parts of the peritoneum would seem to be closely related to, or even evidence of the absorption of the stained oil by and through the lymphatic channels. The disappearance of dye from the oil illustrates the fact that even for a substance as relatively inert chemically as Sudan III, the behavior of dissolved substances cannot necessarily be traced by following the behavior of the solvent oil alone. It was suggested by Dr. C. L. Alsberg that fibrin-like masses observed in rabbits and guinea-pigs and the cyst-like bodies noted in rats and mice presented certain similarities to the calcium and magnesium soap phenomena of the so-called "fat necrosis." This possibility was strengthened by the subsequent finding of gritty material in the cyst-like bodies observed in rats.

The usefulness as a pharmacological method of the intraperitoneal injection of fixed oil as a solvent for fat soluble substances

would seem to be limited, if the entrance of the dissolved material into the circulation is dependent upon the relatively rapid absorption of the solvent oil. This, however, does not imply that its use for administering volatile, serum-soluble and even suspended material might not be advantageous. Until more is known concerning this method, its employment should be accompanied by control experiments in which post mortem examinations are made.

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ON THE INFLUENCE OF COLLOIDS ON THE ACTION OF NON-COLLOIDAL DRUGS. II

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In the first communication (1) one of us proved that rabbit-serum contains substances which are able to inhibit the physiological action of pilocarpine and brought forth arguments in favor of the assumption that the relative insensitiveness of this animal for pilocarpine was due—at least in part—to the presence of these substances in the serum and in the tissues of this animal and we furthermore showed, that the inhibiting power of these substances does not depend upon a chemical destruction of the pilocarpine but on a physical adsorption of the drug. In this communication we want to prove that the same assumptions—and especially the last one—hold good in the case of atropine.

The insensitiveness of rabbits for atropine has been very often investigated by various authors. Willberg (2) showed that rabbits are very insensitive to this drug (242 times less sensitive than human beings and three times less sensitive than the cat) and according to Heckel (3) rabbits may eat larger quantities of atropine-containing leaves without showing any untoward effects.

Fleischmann (4) was the first to draw attention to the fact that the insensitiveness of the rabbit for atropine may be partly explained by the assumption that the blood of this animal is able to destroy atropine. This question was furthermore investigated by Heffter und Fickewirth (5), Metzner (6), Cloetta (7) and others and lastly by Schinz (8). They all were able to confirm Fleischmann's findings, i.e., the blood of most rabbits (there seem to be a few exceptions) can inhibit the action of the atropine. This inhibition was—as far as we can judge—considered to be due to a chemical destruction of the drug though Cloetta and

Schinz described facts which—in view of our later results—show clearly that also in these cases other factors must have played a part. So Cloetta noted, that a solution containing rabbit-serum + atropine and which was only very little active, regained its activity in standing for a considerable time, whereas Schinz described experiments in which was shown that in the mixture of atropine + serum, which is physiologically inert, all the atropine was demonstrable by the “chemical” method of Vitali.

As we had found (1) that the inhibition of pilocarpine by rabbit-serum was caused mainly by physical adsorption we deemed it useful to investigate this matter for the case of atropine. Before being able to do this it was necessary to have a good quantitative method for the physiological determination of the strength of unknown solutions of atropine. Some of the known methods (action on the eye of the cat, antagonistic influence on the muscarin-inhibition of the frog-heart) did not give exact results, so we tried another criterium, viz., the antagonistic action of atropine to the pilocarpine-action on the isolated gut. This method is described in detail in another communication (9). The technique can in a few words be described as follows.

A strip of isolated cat-gut is suspended in Tyrode solution and various doses of pilocarpine are added and washed out again till a dose is found which gives a strong—but still a submaximal—contraction. After this dose of pilocarpine has been in contact with the gut for three minutes a dose of atropine is added and after another three minutes it is recorded whether that dose of atropine has been able to inhibit (partly or entirely) the action of the pilocarpine or not. In this way it is possible to get accurate results presuming that a number of technical details referred to in the paper mentioned above are taken into consideration.

With this method we could confirm the statement of former investigators, that rabbit-serum inhibits the action of atropine in a very considerable degree. Very often we found that a solution of 1 mgm. of atropine in 5 cc. of rabbit-serum showed only one-thirtieth or one-fortieth of the original strength, so that about 96 or 98 per cent of the atropine had been brought into an inactive form.

We now added to a solution of atropine in serum twice the amount of alcohol and filtered. From the filtrate we evaporated the alcohol and by adding water brought the solution to its original bulk. By standardizing this solution on the isolated gut in the manner described above we could show that by treating the serum-atropine solution with alcohol we had regained all the atropine in active form. An example of an experiment as related here is shown in figure 1 a-h.

In figure 1, *a*, first 0.1 mgm. of pilocarpine is added to the solution containing the isolated gut. This dose of pilocarpine gives rather a strong contraction; after three minutes 0.0004 mgm. of atropine is added, which partly inhibits the action of the pilocarpine. In figure 1, *b*, 0.1 mgm. of pilocarpine is added to the same strip of cat-gut and again a strong contraction ensues. Now from a solution of 1 mgm. atropine in 5 cc. of rabbit-serum a quantity is added containing 0.024 mgm. of atropine; this acts more strongly than 0.0004 mgm. atropine did before; showing that the serum had not been able to reduce the atropine action to one-sixtieth of the original strength. In figure 1, *c*, 0.012 mgm. atropine having been in contact with rabbit-serum was added, this had less influence than 0.0004 had before. In figure 1, *d*, 0.016 mgm. atropine of the same serum-solution is given; this acts more strongly than 0.0004 mgm. atropine in a watery solution did before, but somewhat less strongly than 0.0004 mgm. atropine did afterwards (fig. 1, *e*). In this experiment is shown then that the serum was able to inactivate the atropine, so that only one-thirtieth or one-fortieth of the strength was left. This serum now was treated with alcohol in the manner described above and after that practically all the atropine was present again in active form as is shown in figure 1, *f-h*. In figure 1, *f*, and figure 1, *h*, 0.2 mgm. pilocarpine was given to the gut and after three minutes 0.0006 mgm. of atropine was added. In figure 1, *g*, 0.2 mgm. of pilocarpine was added and after three minutes 0.001 mgm. of atropine of the serum-atropine-solution, treated with alcohol. The action of 0.001 mgm. of atropine is a little stronger than that of 0.0006 mgm. of atropine was before, which proves that practically all the atropine is regained in active form.



FIG. 1 a. Isolated cat-gut in 75 cc. of Tyrode solution. 0.1 mgm. of pilocarpine hydrochloride gives strong contraction which is—after three minutes—partly inhibited by the addition of 0.0004 mgm. of atropine sulfate.

b. Antagonistic action of 0.024 mgm. of atropine from solution in rabbit-serum.

c. 0.012 mgm. of atropine from solution in rabbit-serum has only a slight antagonistic action and acts less strongly than 0.0004 mgm. in figure 1, a, showing that more than 29 30 of the active atropine is adsorbed by the rabbit-serum.

d. 0.016 mgm. of atropine acts stronger than 0.0004 mgm. in figure 1, a, but less strongly than in figure 1, e.



e. Control with 0.0004 mgm. atropine in watery solution, showing that the sensitiveness of the gut towards atropine has slightly increased.

f. 0.2 mgm. pilocarpine, after three minutes 0.0006 mgm. of atropine (watery solution).

g. Action of 0.001 mgm. of atropine from a solution in rabbit-serum that is afterwards treated with alcohol as described in text. Shows that by this procedure practically all of the atropine is regained in active form.

h. Control with 0.0006 mgm. atropine, showing that the sensitiveness of the gut towards atropine has not changed during the experiment.

It should be pointed out here, that we did not try to prove that *all* the atropine could be regained by this procedure as we presumed that a part of the atropine would be destroyed chemically, as is shown to happen by Fleischmann, Metzner, Heffter and others. This destruction however does not go very far during the relatively short period of our experiments, whereas the reversible adsorption of the atropine by the serum *does* go to a very considerable degree in a very short time.

In further experiments described extensively in another communication (10) we showed that the adsorption of atropine by rabbit-serum can to a very great extent be inhibited by the addition of sodium citrate and also by adding pepton. Our attention was directed to this fact when we tried to determine the adsorbing power of the whole blood to which we added citrate or pepton in order to prevent clotting. We were rather astonished to find, that this blood had no adsorbing power and found in later experiments that the binding of atropine by serum once having been established could be loosened by the addition of citrate or pepton-solution. We later found, that by the mere addition of water and subsequent boiling we could loosen the binding of atropine + serum to a very large degree, though not so extensively as by the addition of alcohol.

In the first communication we directed attention to the fact that Dixon and Ransom, already in 1912 gave their opinion that the inactivation of alcaloids by animal tissues was not a matter of chemical destruction but was merely brought forth by physical processes so that such a binding could be loosened by physical processes, i.e., addition of water and so forth. They thought that the adsorbing power of rabbit-serum was merely dependent on the "colloidal state" of the liquid. As stated formerly Dixon and Ransom did not give experiments to prove their assumption; but the experiments related here prove that they were right. Only, their opinion as to the colloidal nature being the *only* determining factor seems not to be right in the case of the adsorption of atropine since we found in our experiments (as had indeed been proved by others long before) that many other proteins (cat-serum, human-serum, milk and so forth) had none or only a

slight, adsorbing power for atropine and it seems very improbable to us that this big difference in adsorbing power could be ascribed to differences in "colloidal nature" of the various serums.

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A COMPARISON OF THE ACTION OF CERTAIN DRUGS UPON MUSCULAR WORK IN FROGS

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The object of the investigation was to observe the effects of certain drugs upon the work performed by frogs' muscles under given conditions. The drugs selected were alcohol, cocaine, caffeine and veratrine, and their effects were tested (a) on unfatigued muscle, (b) on muscle whilst fatigue was developing.

a. Unfatigued muscle

Method. All frogs were pithed, brain and cord. Ringer's solution, or the solution whose action was under investigation, was perfused through the muscles in situ from a cannula inserted into the aorta, an outlet being provided by amputation of the ventricle. The left tendo achilles was attached to a myograph lever and free weighted with 10 grams. Electrodes were applied to the origin of the gastrocnemius and to the tendo achilles. Single shocks from a secondary coil were used, the strength of the current being adjusted so that the response of the muscle to the make shock was either just eliminated or just visible on the tracing. In control experiments with a dye added to the perfusing fluid, it was found that the stain did not pass so readily into the gastrocnemius, which was weighted and thus continuously extended, as into the other muscles. This difficulty was got over by releasing the tendon and putting the leg into a flexed position in the intervals between the stimulations. The Ringer's solution was oxygenated and perfused at a pressure of about 15 cm., the pressure being maintained constant by the use of Mariott bottles.

The muscle was stimulated at regular intervals of five minutes, and the response recorded on a moderately quick drum (13 cm. per second).

Ringer. Preliminary control experiments in which plain oxygenated Ringer's solution was perfused showed that continuous perfusion for at least four hours produced little or no alteration in the response to stimulations, repeated at five-minute intervals. The make shock did not elicit any more response than at first, and the break contraction was almost identical in form and extent (fig. 1).

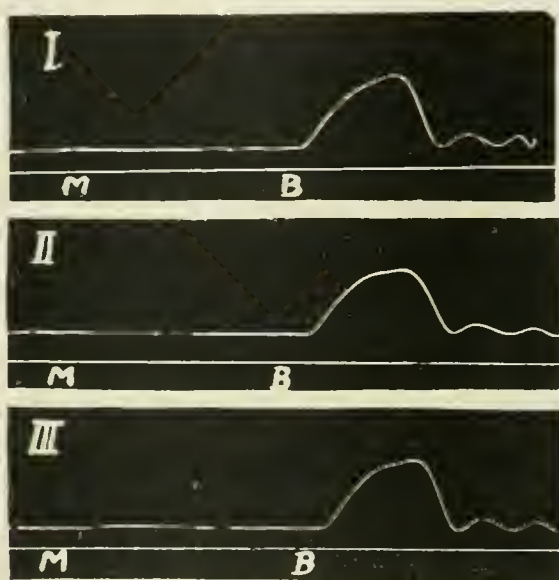


FIG. 1. *M* = make shock; *B* = break shock. I at commencement of perfusion. II after perfusion with oxygenated Ringer's solution for two hours. III after perfusion with oxygenated Ringer's solution for four hours.

Alcohol. The muscle was perfused with oxygenated Ringer containing ethyl alcohol 3 per cent by volume. The height of the break contraction and relaxation period were unaffected by the drug, but there was an increasing response to the make shock. On prolonging the perfusion, this increased response disappeared again (fig. 2).

Cocaine. Solutions containing 0.04 per cent of the hydrochloride were used. The height of the break contraction was diminished, the relaxation period unaffected, and the make response diminished (fig. 3).

Caffeine. Perfusion with solutions containing 0.05 per cent of caffeine showed an increase in height of the break contraction, and an increased response to make (fig. 4). With stronger solutions the relaxation was incomplete and slowed as rigor developed in the muscles.

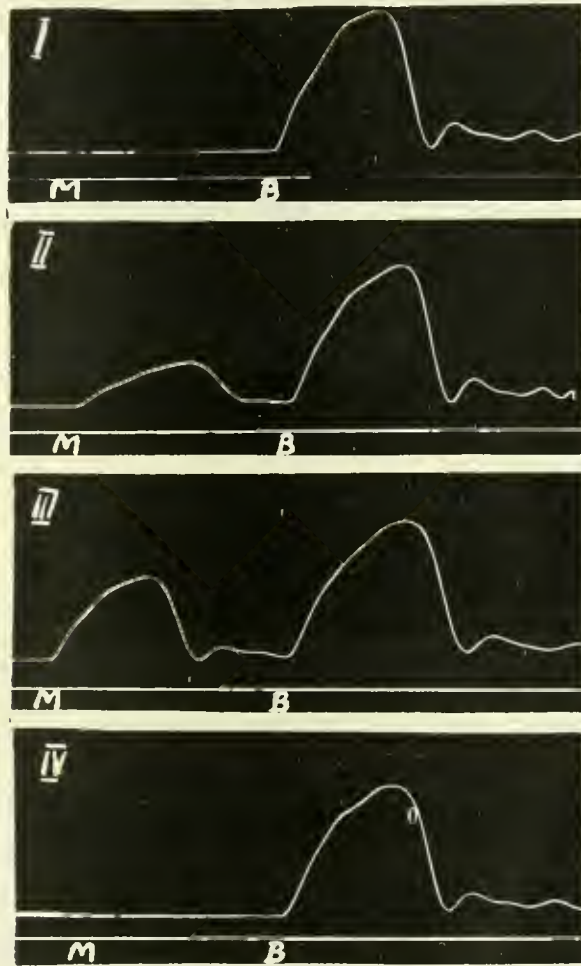


FIG. 2. *M* = make shock; *B* = break shock. I at commencement of perfusion. II after perfusion with Alcohol 3 per cent solution for one-fourth hour. III after perfusion with alcohol 3 per cent solution for one-half hour. IV after perfusion with alcohol 3 per cent solution for one hour.

Veratrine. The solutions used contained 0.001 per cent of veratrine acetate. The height of the break contraction was increased, its relaxation greatly delayed, and the response to make was increased (fig. 5).

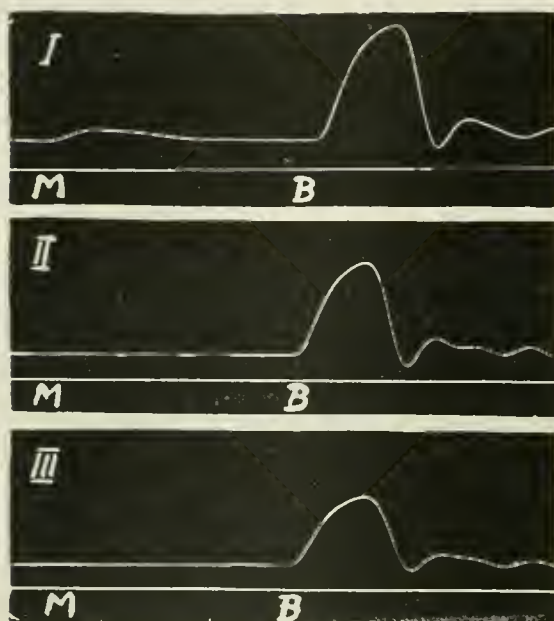


FIG. 3. *M* = make shock; *B* = break shock. I at commencement of perfusion. II after perfusion with cocaine hydrochloride 0.04 per cent solution for one-fourth hour. III after perfusion with cocaine hydrochloride 0.04 per cent solution for one-half hour.

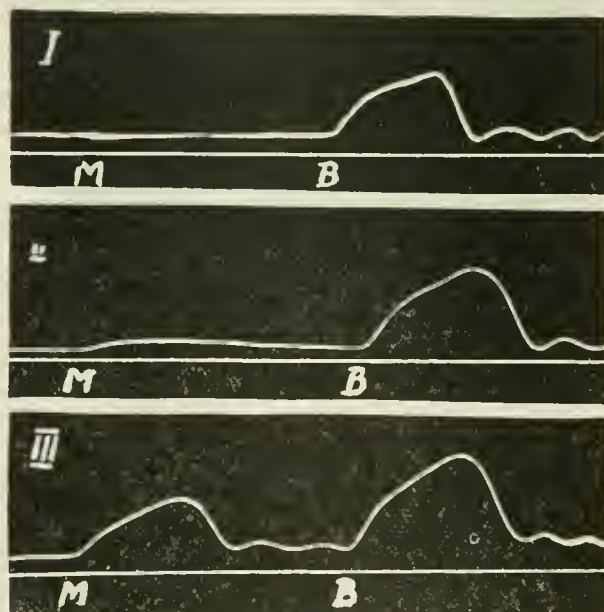


Fig. 4. *M* = make shock; *B* = break shock. I at commencement of perfusion. II after perfusion with caffeine 0.05 per cent solution for one-fourth hour. III after perfusion with caffeine 0.05 per cent solution for one-half hour.

B. FATIGUED MUSCLE

It was hoped that it might be sufficient to compare the corresponding muscles of the right and left leg, but experiments, which were made to ascertain if this comparison were justifiable, proved that the activity of corresponding muscles of the hind legs often differs, both in response to stimuli and in the form of the fatigue curve. This observation confirms that of Crider and Robinson (1).

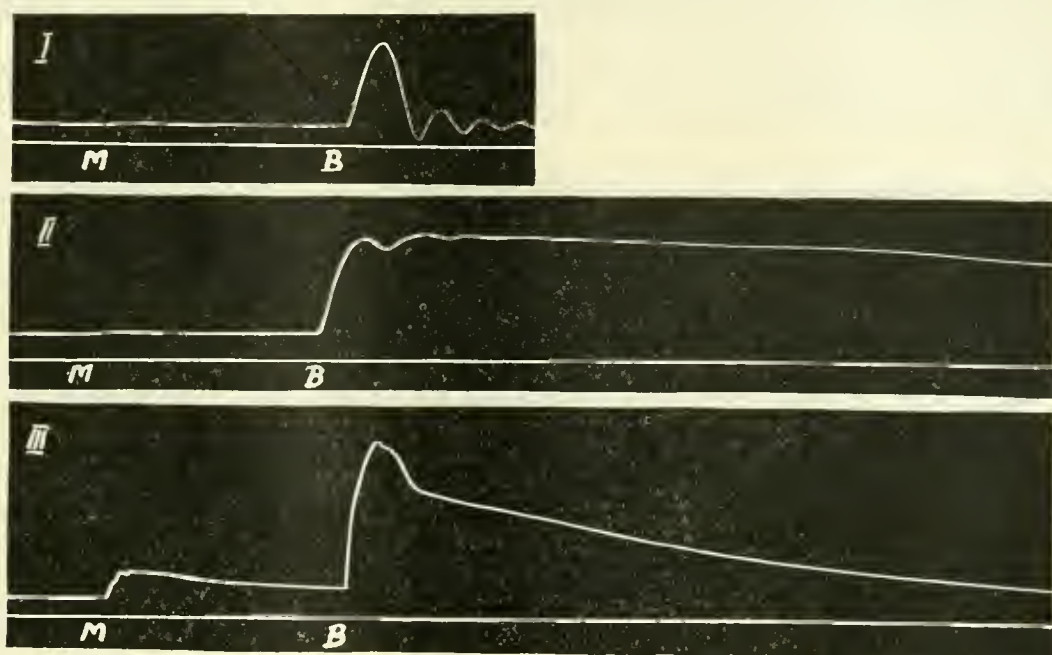


FIG. 5. *M* = make shock; *B* = break shock. I at commencement of perfusion. II after perfusion with veratrine acetate 0.001 per cent solution for one-fourth hour. III after perfusion with veratrine acetate 0.001 per cent solution for one-half hour. In the third curve the drum is rotating at one-fourth its original speed.

This being the case, the triceps femoris was perfused with Ringer's solution and a single fatigue curve recorded, then, as the muscle showed signs of developing fatigue, the perfusion was changed from Ringer's solution to a solution of the drug to be investigated, the stimuli being continued without interruption.

Method. The perfusion was arranged as before, the tendon of the left triceps femoris being attached to the myograph lever, and the muscle free weighted with 10 grams. The sciatic nerve

was cut and placed over the electrodes, and stimulated every three seconds. The break shock of the least current which produced maximal contraction of the muscle was used, and the make shock mechanically excluded. The perfusion fluid was changed during the course of the fatigue curve by means of Mariott bottles. The perfusion pressure was maintained constant at about 40 cm., and the limb arranged at right angles to the trunk (the position of the limb and the high pressure facilitate the passage of the perfusion fluid into the muscle. The fluid can be shown to reach the muscle within a few seconds by adding a dye to the perfusion). The fatigue curve was recorded on a slowly-moving drum.



FIG. 6. CONTROL FATIGUE CURVE; PERFUSION WITH RINGER'S SOLUTION

Ringer. Control fatigue curves were recorded during perfusion of plain Ringer's solution, and they showed no irregularities in the form of the curve (fig. 6).

Alcohol. Five per cent solution by volume of ethyl alcohol, in Ringer, introduced during the course of fatigue, caused an increased response to stimuli and a temporary recovery from fatigue which rapidly passed off (fig. 7). Ten per cent solution of alcohol had a similar but less marked effect.

Cocaine. A 0.1 per cent solution of cocaine hydrochloride caused a more rapidly diminishing response of the muscle to stimulation, and an apparent hastening of the development of fatigue (fig. 8). The fatigued muscle under cocaine did not respond any more readily to direct muscle stimulus than to nervous stimulation.

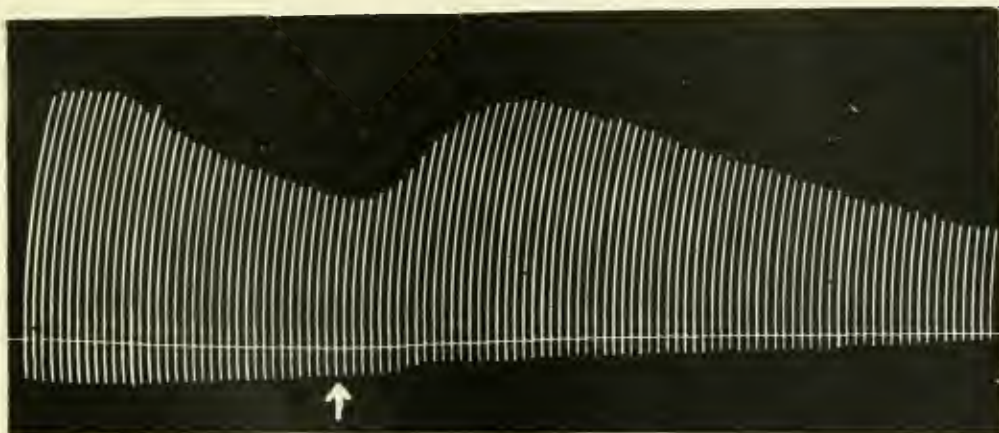


FIG. 7. FATIGUE CURVE; PERFUSION WITH RINGER'S SOLUTION
At \uparrow 5 per cent alcohol added to perfusing fluid

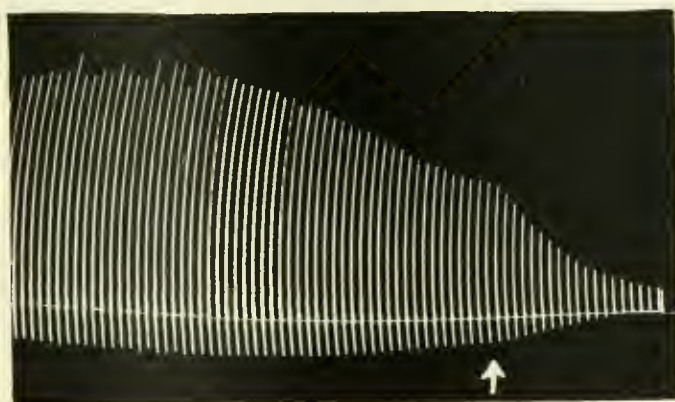


FIG. 8. FATIGUE CURVE; PERFUSION WITH RINGER'S SOLUTION
At \uparrow 0.1 per cent cocaine hydrochloride added to perfusing fluid



FIG. 9. FATIGUE CURVE; PERFUSION WITH RINGER'S SOLUTION
At \uparrow 0.25 per cent caffeine added to perfusing fluid

Caffeine. On changing the perfusion fluid to a solution containing 0.25 per cent of caffeine the curve showed a slight temporary recovery from fatigue (fig. 9).

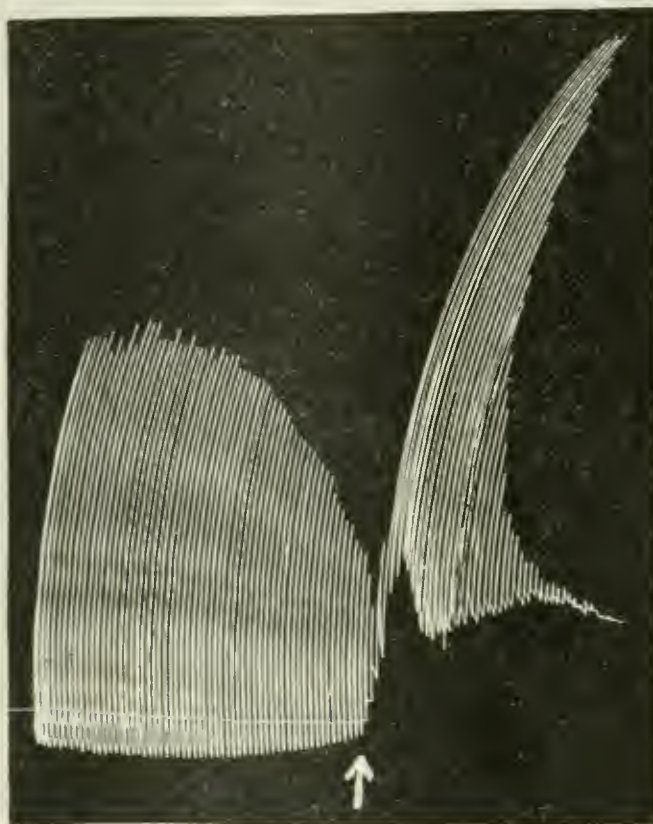


FIG. 10. FATIGUE CURVE; PERFUSION WITH RINGER'S SOLUTION
At \uparrow 0.01 per cent veratrine acetate added to perfusing fluid

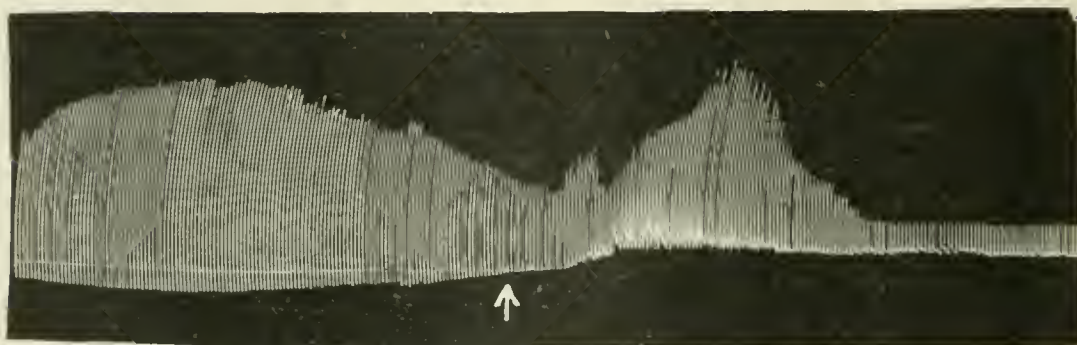


FIG. 11. FATIGUE CURVE: PERFUSION WITH RINGER'S SOLUTION
At \uparrow 0.01 per cent veratrine acetate added to perfusing fluid

Veratrine. A 0.01 per cent solution of veratrine acetate had the most marked effects on the course of the fatigue curve. The muscle response to stimulation was increased even beyond the original maximal contraction (fig. 10). There was a temporary recovery from fatigue, sometimes with apparent delay in the production of complete fatigue (fig. 11). Also the relaxation of the muscle was incomplete and slowed.

DISCUSSION

The results shown in the two sets of tracings are mutually confirmative.

Alcohol causes primarily an increase in the excitability of the muscle (indicated by increased response to the make shock) accompanied by a temporary increase in the work done. This is followed by lessened excitability and diminished response. That the effect is not due to increase of energy derived during the assimilation of alcohol seems to be shown by the rapidity with which the effect develops and passes off in the course of the fatigue curve. Effron found similar effects on muscular contraction after the application of alcohol to the nerve only in a muscle-nerve preparation (2).

Cocaine has a directly depressant effect on striated muscle. The course of the fatigue curve after introduction of cocaine shows a marked and rapid fall. The effect is not entirely due to the action of the drug on nerve fibers, for direct application of the electrodes to the fatigued muscle elicited no further response, whereas stimulation of the opposite sacral nerves (equally exposed to the action of the drug) produced a normal contraction in unfatigued muscles. Under these conditions, therefore, fatigued muscle is depressed by the drug before any marked effects have developed in nerve fibers or in unfatigued muscle. The experiments of Kuroda demonstrate a direct depressant action of cocaine on plain muscle (3). The reported effect of coca leaves, in increasing the power to do muscular work, must therefore be due to the action of the drug on the central nervous system. These results confirm those of Kubota and Macht (4),

obtained by the use of different methods, and would seem, therefore, to be conclusive.

The effects of perfusion of caffeine with single shock stimuli indicate a distinct increase in excitability and response of the muscle, and during prolonged perfusion there is a permanent rise in muscle tone with, ultimately, development of rigor. In the fatigue curve these effects result in some increase of the work done, and the comparatively slight effect suggests that the increased power of doing muscular work, under the influence of caffeine, is due to its central rather than its peripheral action. The direct action of caffeine upon unfatigued muscle is more marked than its action upon muscle which is developing fatigue.

Veratrine extraordinarily increases the excitability and response of muscle, and there is an accompanying rise of tone as shown by the single shock tracings. Its effect on the fatigue curve is very marked indeed. At first there is an enormous increase in the work done, followed by a rapid diminution in excitability.

Chemically the four substances are very different, and it is difficult to explain their action upon striated muscle on the ground of any similarity of chemical properties. In basicity, for example, veratrine is intermediate between alcohol which is a neutral substance, and caffeine which is a moderately strong base. Nor do the effects depend only on the rate of perfusion through the muscle, as influenced by the action of the drugs on the calibre of blood-vessels, for although cocaine constricts and caffeine and alcohol dilate the vessels, yet veratrine, which constricts vessels, has the most marked effect in the direction of increasing muscle work.

It is hoped, in a further communication, to make some attempt to elucidate the action of these drugs.

SUMMARY

1. The action of a drug on striated muscle is shown more conclusively by comparison with previous conditions in the same muscle than by the use of two corresponding muscles, since these are not necessarily equally responsive.

2. The direct action of alcohol and veratrine on frog's muscle is to increase temporarily the amount of work done.

3. Cocaine directly depresses striated muscle without any preliminary increase in response.

4. On muscle which is becoming fatigued, caffeine has very slight restorative action, although an obvious increase in excitability occurs in unfatigued muscle.

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THE ACTION OF HISTAMINE AND PEPTONE ON THE ISOLATED SMALL INTESTINE

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This work was originally intended as a part of an investigation concerning the underlying causes of paralytic ileus. Under the influence of Magnus' theory, that the pendulum movements as well as the peristalsis are dependent upon Auerbach's plexus, I thought it justifiable to assume, that a certain parallelism exists between the two forms of intestinal motor activity with regard to their reactions to drugs and other influences. Work along other lines of the same problem however soon led to the conviction that no such parallelism exists and that in fact the pendulum movements and the peristalsis may react entirely differently to the same influence. If, for instance, the intestines are handled gently in the air for half an hour, the peristalsis as shown by Cannon and Murphy (1) will be inhibited for several hours. On the other hand this manipulation is wholly without effect upon the pendulum movements. This is easily demonstrated if a cat is given a bismuth meal and then manipulation of the intestines immediately started. Even during the manipulation vigorous rhythmic contractions are very frequently observed, and if afterwards the contractions are recorded in a saline bath or with Trendelenberg's (2) modification of the saline bath method, they will be seen to go on in the usual manner for several hours. If the recording of the pendulum movements is discontinued after two to three hours and an x-ray observation made, it will be seen, that nothing has passed out of the stomach. This manipulation of half an hour is by no means the limit of what the pendulum movements will stand in the way of mechanical injury; even two hours of vigorous handling will not abolish

them. Inhibition of the peristalsis on the other hand is manifest after a trauma much less severe than half an hour of manipulation. This difference in reaction to mechanical injury is too striking not to raise doubts as to the soundness of the neurogenic theory of the pendulum movements. It is hard to conceive, that the two motor functions of a relatively simply constituted nervous system like Auerbach's plexus, should react so fundamentally differently to mechanical traumatization, and evidently this observation is much more satisfactorily explained by Bayliss and Starling's (3) opinion, that only the peristalsis is governed by Auerbach's plexus, but that the pendulum movements are of myogenic origin. Although the neurogenic theory of Magnus (4) has been almost universally accepted, some recent observations speak strongly in favour of the view of Bayliss and Starling. Gunn and Underhill (5) were able to get ganglion free strips of the cat's small intestine to contract rhythmically, and they also found, that the rhythmic contractions could be revived as long as five days after the death of the animal. This exceeds every known limit for the survival of ganglion cells and seems to exclude the possibility of neurogenic origin of the pendulum movements.

In the isolated intestine only the pendulum movements are observed, and evidently no conclusions can be drawn from the observations of the action of histamine and peptone under these conditions with regard to the action of these substances on the peristalsis, or their possible importance in the bringing about of intestinal paralysis. As however these experiments have some interest from a pharmacological and physiological point of view, a brief publication of the results seems to be justified.

It is well known that histamine has a strong stimulating effect on smooth muscles and consequently also on the rhythmic contractions of the isolated intestine. The object of this investigation was to find out if a paralyzing effect could be obtained if the concentration of histamine was sufficiently high. It is reasonable to expect a similar effect, because it is a biological law, that a substance, which in low concentrations has a stimulating effect on a tissue, will paralyze it in high concentrations. That this also holds good for histamine has recently been shown

by Abel and Macht (6), who were able to paralyze the uterus or the guinea-pig with histamine in a concentration of 1:1087.

Peptone has not been much studied with regard to its effect on the intestine. It has been found by Roger (7) to have a stimulating effect on the intestine of the rabbit. Dale and Laidlaw (8) have shown peptone to have a stimulating effect on smooth muscle, and in fact found such a close correspondence between the pharmacological properties of peptone and histamine as to suggest the presence in peptone of histamine, or at least of a substance related to it.

TECHNIQUE

Cats and rabbits were used for the experiments. The cats were killed by etherization and cutting out the heart, the rabbits by a blow in the neck. The abdomen was then immediately opened, a loop of the small intestine removed and washed out with Ringer's or Tyrode's solution. For rabbits Tyrode's solution was always used, for cats except in a few instances Ringer's solution. The intestine was then placed in a beaker containing Ringer's or Tyrode's solution and during the time of the experiment was kept in an ice box at a temperature of approximately 8° to 10°C. In the case of the rabbit's intestine it was found advisable to keep the intestine supplied with oxygen. Otherwise there was usually a loss of excitability and in the power of the spontaneous contractions after three to four hours, whereas if a sufficient oxygen supply was maintained excellent preparations could be obtained seven to eight hours or even more after death. The cat's intestine is much more resistant than the rabbit's under the above conditions, and provided that the loop was carefully washed out and kept at a low temperature, good preparations could often be obtained twenty-four hours after the death of the animal. To keep on the safe side however no preparation older than twelve hours was ever used, and in the majority of the experiments the preparations were not older than three to four hours.

The technique in recording the contractions of the intestine was in principle the same as devised by Magnus (9) and modified

for pharmacological purposes by Neukirch (10). In all cases the contractions of the longitudinal muscle layer were used for recording. A strip of 2 cm. in length and approximately 3 mm. wide was prepared and suspended in a test tube of the same kind as described by Neukirch and containing 75 cc. of Ringer's or Tyrode's solution. The temperature was kept constant at 38° to 40°C. by means of a thermo regulator and a steady stream of oxygen bubbles entered at the bottom of the test tube. Through another tube also entering at the bottom of the tube the fluid could be changed without disturbing the preparation. One end of the strip was fastened to a small hook at the bottom of the test tube and the other end was connected to a muscle lever, which recorded the contractions on a slow moving smoked drum. The speed of the drum is the same in all the figures reproduced or approximately 5 to 6 mm. in the minute.

The histamine used was the bichloride of the amine manufactured by the Hoffman La Roche Company in New York. The solutions were always made up fresh and no solution older than twenty-four hours was used. The histamine was dissolved in 1 to 5 cc. of distilled water. Control experiments showed that the dilutions of a bath volume of 75 cc. of Ringer's solution with 5 cc. of distilled water did not in any way affect the contractions of the strip.

The peptone used in these experiments was a peptone made for bacteriological purposes by Fairchild Brothers and Company, New York. Its chemical character is indicated by the following reactions. It was readily soluble in ten parts of distilled water. Boiling caused no coagulation, but precipitation occurred on adding 95 per cent alcohol, nitric acid at ordinary temperature and sodium chloride to saturation. The precipitate with nitric acid disappeared on warming but reappeared on cooling.

Evidently the preparation is far from being a peptone in the chemical sense of the word, but is rather to be regarded as a mixture of hydrolytic cleavage products of proteins, which partly at least must consist of compounds giving the reactions of proteoses.

The peptone solutions also were made up fresh each time, and usually a 10 per cent solution in Ringer's solution was used.

I. HISTAMINE EXPERIMENTS

a. Intestine of cat

The general type of contractions exhibited by the cat's isolated small intestine is well known and need not be repeated here. It is generally contended that two different kinds of contractions can be recognized: First, rhythmic beats of great regularity and occurring at a rate of 10 to 14 beats in the minute, and secondly, tonus variations of a much slower and irregular periodicity. This perception of two different kinds of contractions is of interest in this connection because histamine seems to have a somewhat different effect on the two modes of contraction. This is not the case with histamine only. According to Magnus (11) the tonus variations are abolished by atropine, which however leaves the rhythmic beats undisturbed, and the curve after a small dose of atropine takes an aspect of much greater regularity than normally.

Although it is frequently difficult and to a certain extent arbitrary to decide in a strongly stimulated intestine, which of the contractions are to be classified as tonus variations, and which are rhythmic beats, I have in the following endeavored to make this distinction because in many cases the difference in the action of histamine on the two types of contraction is too striking to be ignored, and it seems to be the most logical course to extend this distinction also to the cases where the difference between the two kinds of contraction is not so apparent. As a general rule I have characterized the slow powerful contractions seen after histamine poisoning as tonus variations, and contractions superposed on them and approximately resembling the rhythmic beats in rate and amplitude as such.

As this paper deals mainly with the effect of large doses of histamine, no attempt was made to determine the effect of very small doses. It is well known, however, that the smooth muscle of the intestine is highly susceptible to histamine, and that a distinct response is obtained with dilutions of one to several millions.

The lowest concentration of histamine used in this work was 1:750,000, and experiment 13 (fig. 1) may be regarded as a typical example of the effect of this concentration. Before histamine was given the rhythmic beats occurred regularly at a rate of approximately 10 to 12 contractions in the minute. Immediately after histamine was added a very strong rise in tonus occurred. This rise in tonus did not in any way interfere with or influence the rhythmic contractions, which are seen going at the same rate and amplitude as previously. The tonus remained steady at the same high level for approximately ten minutes and then gave away to a series of very strong tonus variations. The rhythmic contractions were not materially affected except that, as seen also normally when the tonus variations are well pronounced, the contractions often did not

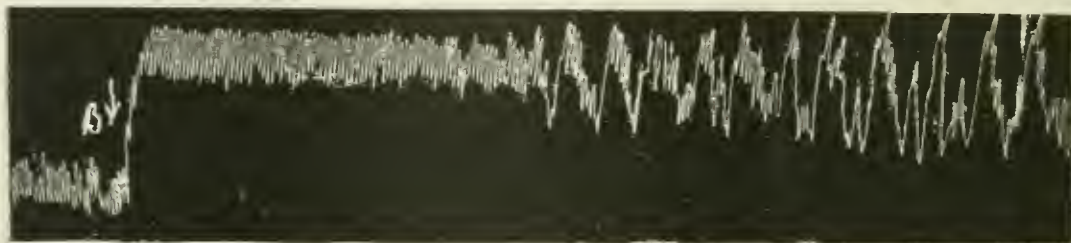


FIG. 1. SMALL INTESTINE OF CAT

Tyrodes solution, 38°C. At *b* histamine 1:750,000

relax completely when the tonus is rising, but a new contraction started when a partial relaxation had taken place. Exactly the opposite process is seen when the tonus wave is on its downward movement; the relaxation is complete, but the following contraction does not reach its full height before it is overcome by a new relaxation. For about half an hour the tonus variations continued with the same strength, but then gradually became weaker and more irregular and finally, one and one-half hours after the addition of histamine the contractions had resumed perfectly the same type as had prevailed before histamine was given. This gradual giving away of the histamine effect is a quite typical occurrence and is seen after all concentrations of histamine, except perhaps the very large, which completely paralyze the intestine. It must be due to either a loss of respon-

siveness to histamine by the structures affected by it or to a disappearance of histamine from the bath fluid. This point will be discussed later on.

In figure 2, *a*, is shown the effect of a histamine concentration ten times as large or 1:75,000. A strong increase in tonus followed immediately and the tonus remained high for a few seconds. The tonus then slowly fell and at the same time slow powerful contractions of the type of tonus variations occurred. If figures 1 and 2, *a*, are compared the relative tonus increase caused by the

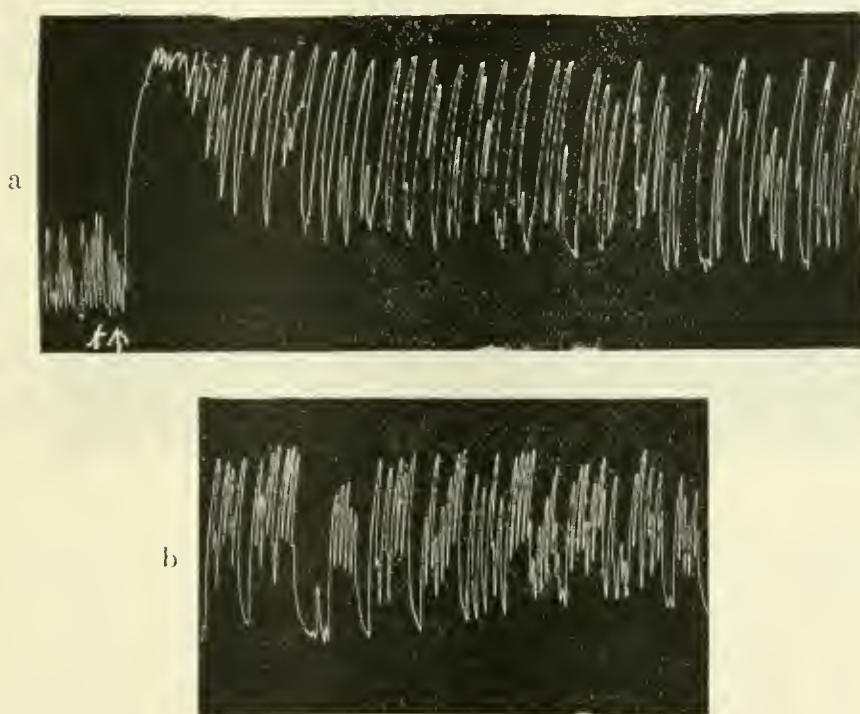


FIG. 2. SMALL INTESTINE OF CAT

Tyrodes solution, 38°C. At *a* histamine 1:75,000

histamine is about the same, and the chief difference between the two tracings is in connection with the rhythmic beats. In figure 1 we see these go on practically undisturbed, whereas in figure 2, *a*, the rhythmic beats seem to be entirely suppressed and the appearance of the curve is dominated by the tonus variations. Almost all of the contractions seen on this curve are of the slow powerful type and agree in their aspect much more with the tonus variations, than with the rhythmic beats. As the histamine effect passes off however, the tonus variations

gradually decrease in amplitude and frequency and rhythmic beats appear again superposed on the tonus variations. About forty-five minutes after the addition of histamine, the rhythmic beats have resumed almost their previous rate and are perhaps slightly increased in amplitude, but the tonus variations were still considerably stronger, than they were before the histamine was given (figure 2, *b*). After a little more than an hour the entire effect had passed off, and the curve had the same aspect as previously.

The interpretation of the contractions seen in the tracing reproduced in figure 2 can of course not be regarded as proven. It is mainly a suggestion founded upon the much closer conformity of the contractions with tonus variations than with rhythmic

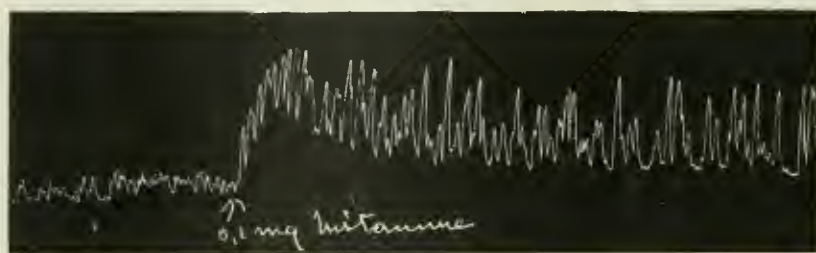


FIG. 3. SMALL INTESTINE OF CAT

Ringer, 39°C. The strip contracted poorly. Marked improvement after addition of 0.0001 gram histamine. Bath volume 75 cc.

beats with regard to their amplitude, rate and time of evolution. The reappearance of small contractions, in rate and amplitude agreeing with the rhythmic beats, as the tonus variations decrease also supports this suggestion. From figure 1 it is so evident, that in this contraction histamine mainly stimulates the tonus variations, that the interpretation given to figure 2 seems justified. Naturally this conception of the relationship of the rhythmic beats and tonus variations must not be carried too far. Even if there exists a certain degree of independence between the types of contraction, there can be no doubt that they are also in numerous ways dependent upon each other. It is also by no means always true that the stimulating effect of histamine is confined to the tonus variations. In many cases there is a distinct stimulation of the rhythmic beats too, although

this never is so marked as the stimulation of the tonus variations and is most frequently seen in cases where the activity of the intestine is poor before addition of histamine. An example of this may be seen in experiment 17, figure 3. Here the strip contracted poorly with a slow rate and very weak contractions. Histamine in a concentration of 1:750,000 caused a tonus increase, stimulation of the tonus variations and a marked increase in the amplitude of the rhythmic beats. In this case it was particularly difficult to distinguish between tonus variations and rhythmic beats after the stimulation by histamine and it may be gathered from this experiment, that in many cases this distinction has

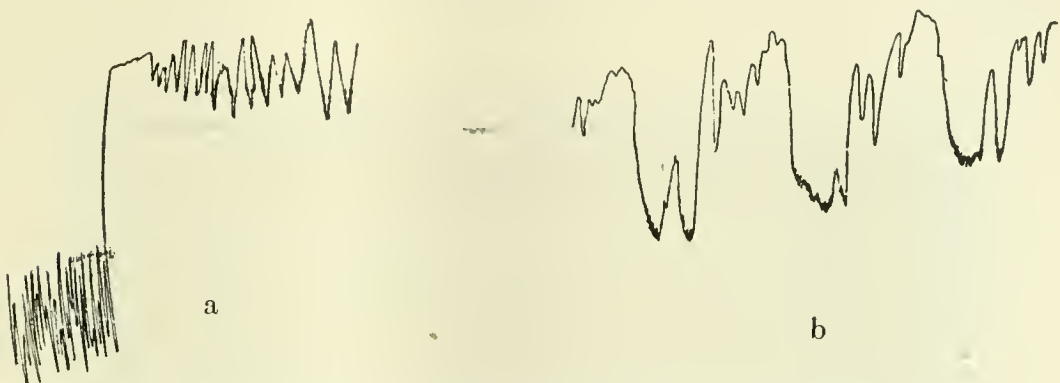


FIG. 4. SMALL INTESTINE OF CAT

Ringer, 39°C. At *a*, histamine 1:4000. *b*, Same strip as *a*, fifteen minutes after the addition of histamine. (Tracings of figure 4 *a* and *b* were badly broken in the mail, but have been faithfully traced and photographed.)

only a limited value as an aid in the interpretation of the effects of histamine.

In experiment 26 (figure 4, *a* and *b*), there can be no doubt, that the contractions seen after histamine had been given are of the type of tonus variations. The concentration of histamine used in this case was 1:4000. A very marked rise in tonus followed the addition of histamine and the intestine remained in a state of tonic contraction for about five minutes. During this time small slow contractions occurred and as the tonus gradually fell, these contractions became correspondingly larger. Ten minutes later they had the character of strong tonus variations, each occupying a time of approximately two to three

minutes (figure 4, *b*). The rhythmic beats at first were almost entirely suppressed but gradually as the tonus variations decreased in strength, they reappeared and half an hour after the addition of histamine very definite rhythmic contractions were superposed on the tonus variations. The rhythmic beats continued to grow in frequency and amplitude, while the tonus variations almost entirely stopped forty-five minutes after the addition of histamine. Instead the intestine remained in a very high state of tonus, which showed a tendency to rise further. This is a rather unusual effect because generally the tonus falls to the pre-histamine level as the histamine effect passes off. The strip was washed out with Ringer's solution about two hours after the histamine had been given. The rhythmic beats at this time had a nearly normal rhythm and amplitude, but the general tonus still remained very high and the strip appeared to be in a state of tonic contraction. Washing out failed to restore the strip to its previous state of tonus although approximately 1000 cc. Ringer was used for the wash out. Generally however the wash out of the strip promptly initiates recovery from the histamine effect if a moderate or comparatively small dose, as for instance 1:25,000 is given. There seems also to be some individual variability in the power of recovery after changing the fluid, just as different strips show a different susceptibility to the effect of histamine.

In experiment 29 (figure 5) a concentration of 1:2000 was used. An extremely strong rise in tonus followed immediately on adding the histamine, but the tonus then fell rapidly again to the previous level and all contractions were completely inhibited for four to five minutes. Then the strip began to contract again, at first slow contractions of the type of tonus variations although comparatively very weak, and these soon became superposed by rhythmic beats. Fifteen minutes after the addition of histamine the curve had almost the same aspect as before; the only difference being that the tonus variations were a little more pronounced. In this concentration histamine evidently caused first a stimulation and then an inhibition of the contractions. When the contractions started again, they had almost

the normal rate and amplitude, and it seems, that the most probable explanation is, that in this case the stimulating and inhibitory effects of histamine about balanced each other, so that the visible result is hardly noticeable.

In order to confirm this interpretation experiment 27 (fig. 6, *a*) was performed. In this experiment 0.5 mgm. histamine was added to the bath at intervals of two to three minutes. The first dose giving a concentration of 1:150,000 gave the usual effect; a strong rise in tonus and a series of strong tonus variations. The tonus variations continued to increase in strength after each subsequent dose of histamine until a concentration of about 1:15,000 had been reached. From now on each subsequent dose

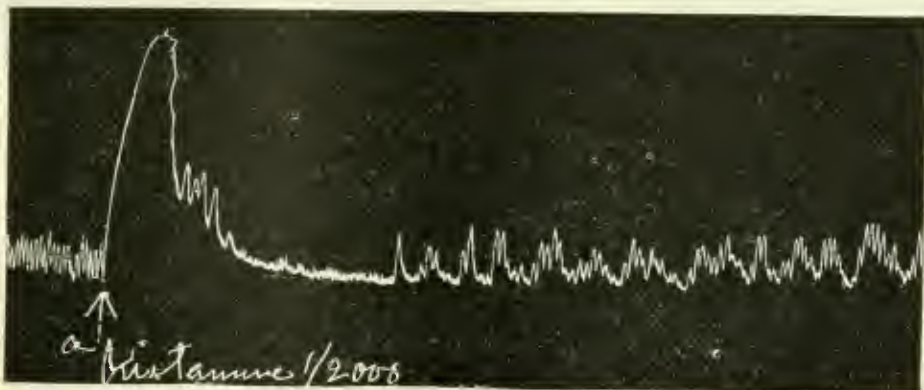
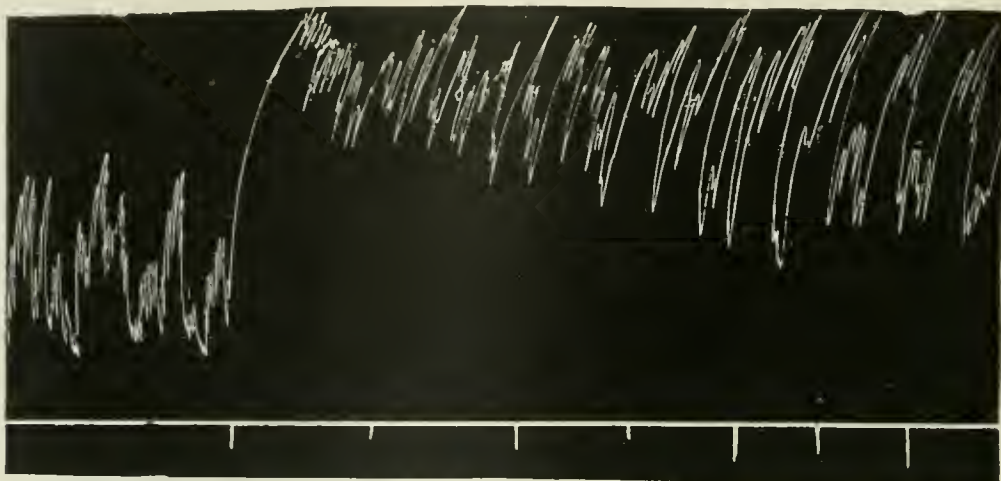


FIG. 5. SMALL INTESTINE OF CAT
Ringer, 39° to 40°C. At *a*, histamine 1:2000

caused a slight but definite drop in tonus and in the amplitude of the tonus variations. It might however be suspected, that this drop in tonus was due simply to the usual passing off of the histamine effect, and a predominance of the tendency of the intestine to resume its normal type of action. For this reason 11 mgm. histamine were given in one dose, thus increasing the concentration to 1:2143 (fig. 6, *b*). The drop in tonus was quite marked and the amplitude of the contractions was now even smaller than before the first dose of histamine. If allowance is made for the decrease in power of the contractions, which in many cases follows when the intestine has been suspended in the test tube for an hour or so, it can be said, that after the last

dose of histamine the stimulating and inhibitory effects of histamine about balance each other, and so confirm the interpretation given to the tracing reproduced in figure 5.



a



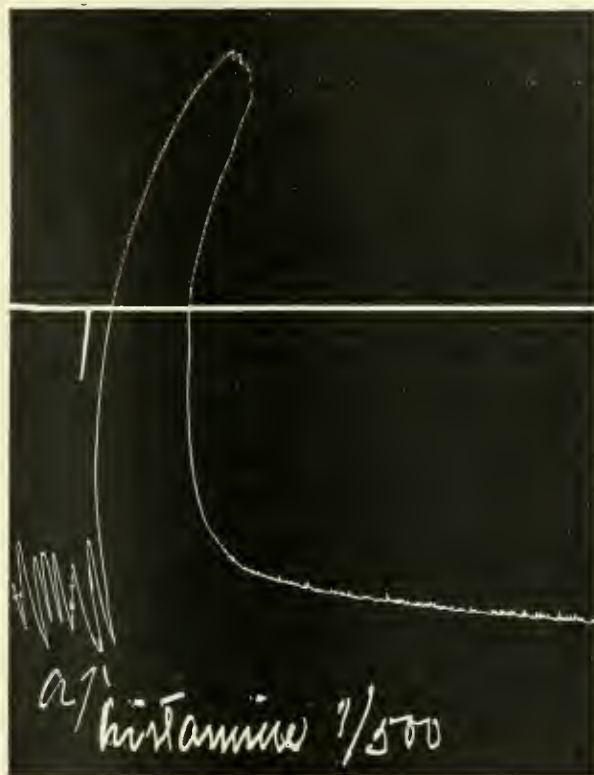
b

FIG. 6. SMALL INTESTINE OF CAT

a, Ringer, 39°C. Bath volume 75 cc. At each mark on the bottom line 0.0005 gram histamine was added to the bath. *b*. Same strip as *a*, forty minutes after the first dose of histamine had been given. Histamine is still being added to the bath every two or three minutes in doses of 0.0005 gram. At the last mark on the bottom line 0.011 gram histamine was given in one dose.

In experiment 42 (fig. 7, *a*) a concentration of 1:1500 was used. In this concentration of histamine the inhibitory effect is absolutely predominating. An extremely strong rise in tonus followed immediately after histamine had been given, but the

tonus rapidly fell again, and the intestine remained relaxed and perfectly motionless for two and one-half hours. At the end of this time occasional very weak contractions were seen, perhaps



a



b

FIG. 7. SMALL INTESTINE OF CAT

Ringer, 39°C. At *a*, histamine 1:500. *b*. Same strip as *a*. This strip had remained paralyzed for two and one-half hours. Between *d* and *e* the strip was washed out with 700 cc. warm Ringer's solution. Contractions started almost immediately after the washing had begun.

indicating that the strip was on its way to recovery. After washing out with 700 cc. Ringer the strip recovered, and the contractions now had the type usually seen when a large but not paralyzing dose of histamine has been given (fig. 7, *b*). A second wash out with 500 cc. Ringer did not materially change the type of the contractions. Evidently it is not possible with fluid volumes used in this case completely to remove all the histamine.

In every case where histamine had been given, there was a tendency of the intestine to recover from the histamine effect. The time necessary for recovery varies according to the amount of histamine given, and after small doses there is usually a complete recovery after an hour or two. When very large doses are used the recovery is slow, but the tendency of the intestine can be clearly seen after concentrations of 1:2000 to 1:4000. Two factors may be responsible for the recovery from the histamine effect, either a destruction or conversion of histamine in the bath into some inactive compound, or a decreasing sensibility of the intestine to the effect of histamine. The first of these suggestions seemed rather improbable since Mellanby (12) has stated, that histamine, when injected into a loop of intestine in the living animal, does not disappear in the course of four to five hours if the resorption from the loop is prevented by ligation of the vessels. As the experimental conditions are different, it seemed advisable to determine whether any appreciable amount of histamine disappears from the test tube during the course of three to four hours. The height of contraction of a guinea-pig's uterus was used to compare the amount of histamine present in the bath fluid three hours after the histamine had been added to the bath containing a strip of intestine, with the effect of a histamine solution freshly made up and of a known strength. The fluid to be tested was diluted so as to contain histamine in a calculated concentration of 1:15,000,000. Estimated in this way there was exactly the same amount of histamine in the bath fluid as theoretically should be there.

From these experiments it is evident, that histamine in sufficient large doses has a paralyzing effect on isolated strips of the cat's small intestine. It seemed to be of some interest to find

out if this inhibition of the contractions could be obtained if histamine is allowed to act on the intestine only from the mucous membrane, that is if histamine is injected into an isolated loop of intestine.

To this end a loop of intestine was removed immediately after death of the animal, tied off in both ends and a fine needle inserted through the intestinal wall near one end. The length of the loop was approximately 10 cm. Two points on the middle of the loop were fixed by two small hooks 3 cm. apart to a glass rod at the bottom of a beaker containing Ringer's solution. Another point of the intestine, 3 cm. from one of these hooks was connected to a muscle lever. The solution was oxygenated in the usual manner. The curves resulting from this arrangement showed the same general type of contractions as the isolated strips, although they were not quite as regular, as naturally the contractions of the circular muscle layer interfered more with the contractions of the longitudinal layer than in the case of an isolated strip.

Two experiments of this kind were performed. In one case 32 mgm. and in another 100 mgm. histamine were injected into the loop. In the first case only a stimulating effect was obtained, and the curve had very much the same aspect as that written by an isolated strip after a moderate dose of histamine. In the second case where 100 mgm. were injected, there was first a very strong stimulation of the usual type, but this stimulation did not persist more than about fifteen minutes, and after this time there was rather an inhibition than a stimulation as compared with the amplitude and frequency of the contractions before histamine was injected. Usually the stimulation brought about by histamine lasts considerably longer, and the best explanation of the short duration in this case seems to be, that when the histamine has become evenly distributed throughout the loop, which distribution might reasonably be expected to take place in the course of ten to fifteen minutes, the concentration of histamine in the loop is such as to make its stimulating and inhibitory powers balance each other.

It will be recalled, that this interpretation was offered for the almost negligible effect of histamine in a concentration of 1:2000 in experiment 29 (fig. 5). One might therefore expect, that as in the case of an isolated strip, histamine in a sufficiently large dose will have a paralyzing effect when injected into an isolated loop. This lack of response to histamine is not peculiar to the intestine. Abel and Macht (13) made similar observations on the uterus of the mouse and the guinea-pig. They found, that on increasing the strength of the histamine solution the stimulating effect disappears and within certain limits of concentration there is no response at all. On a further increase the uterus responds with relaxation and inhibition of the contractions.

b. Intestine of rabbit

The normal contractions of the rabbit's intestine when suspended in Tyrode's solution present some differences from those of the cat's intestine under the same conditions. The tonus variations are not at all so marked, and we have a sequence of very regular contractions of almost identical amplitude. There are also some marked differences in the effect of histamine. The stimulating effect of histamine is not at all so marked in the rabbit's intestine as in the cat's. The individual variability in the strength and nature of the response also seemed to be greater.

In figure 8 it will be seen that histamine in a concentration of 3:750,000 did not have any effect at all, and the increase of the concentration to 1:75,000 had a distinct inhibitory effect. In another experiment (fig. 9) the intestine reacted upon a concentration of histamine of 1:30,000 with a good increase in tonus. As the effect of large doses of histamine was the main object of this investigation, no further attempts were made to analyze the significance of these and similar observations.

Large doses of histamine, concentrations of 1:4000 and upwards, always caused a strong initial stimulation, but this stimulation invariably was of much shorter duration than in the cat and the stimulation usually passed off in a few seconds. This stimulation was always followed by a more or less marked



FIG. 8. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. Bath volume 75 cc. At *a*, histamine 1:75,000. As this had no visible effect 0.001 gram histamine was added at *b*, thus making a concentration of histamine of 1:75,000.



FIG. 9. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. At *a*, histamine 1:30,000

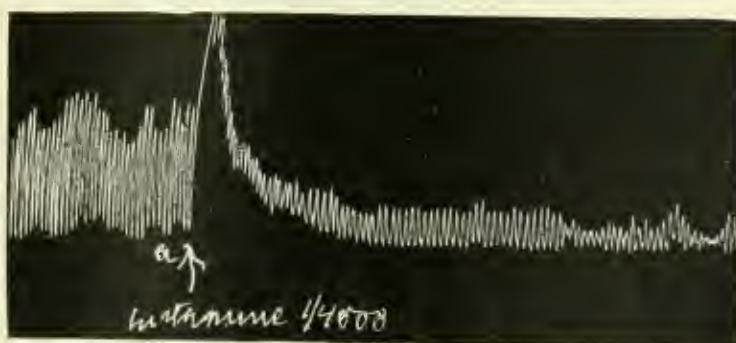


FIG. 10. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. At *a*, histamine 1:4000

inhibition, which when the concentration was sufficiently high amounted to total paralysis. The power of recovery is also less in the rabbit than in the cat. In figure 10 the effect of a concentration of 1:4000 is seen. The stimulation following immediately after the addition of histamine to the bath passed entirely off in two minutes, and the following contractions were

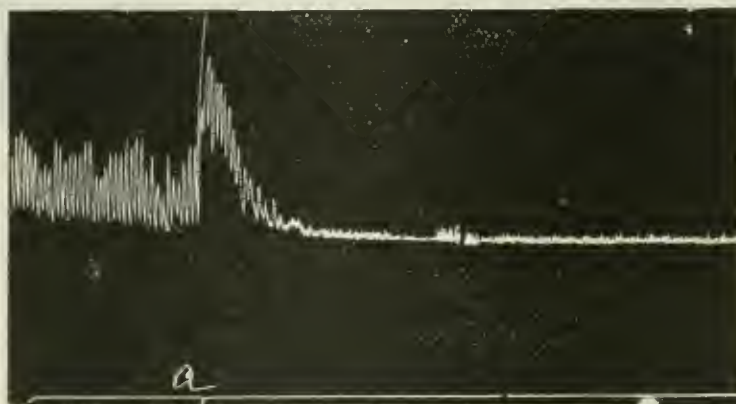


FIG. 11. SMALL INTESTINE OF RABBIT

Tyrode, 38° to 39°C. At *a*, histamine 1:738. The small oscillations seen on the curve after the strip had relaxed are caused by the oxygen bubbles.

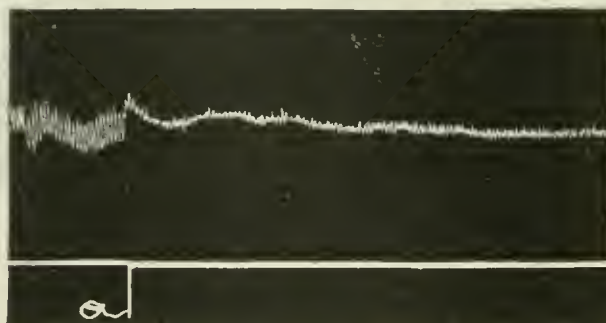


FIG. 12. SMALL INTESTINE OF RABBIT

Tyrode, 40°C. At *a*, histamine 1:950. The small oscillations on the curve after relaxation of the strip are due to the oxygen bubbles.

much reduced both in amplitude and frequency. No tendency to recovery was seen and the contractions continued to decrease slowly during the two hours the experiment was going on. A complete inhibition of the contractions occurred if concentrations of 1:700 to 1:900 were used. In experiment 31 (fig. 11) a concentration of 1:738 was used. The initial stimulation followed

by a complete inhibition, which lasted for about fifteen minutes. Then the strip gradually began to recover, but the contractions never reached their previous frequency and amplitude. A complete inhibition was elicited in experiment 30 (fig. 12) by a concentration of 1:950, and here no recovery was observed as long as the experiment was continued or thirty minutes. An increase of the concentration of histamine, ten minutes after the first dose had been given to 1:725 had no visible effect.

With allowance for individual variations it can be said, that complete inhibition of the rabbit's small intestine is obtained with approximately the same concentrations of histamine as for the cat's intestine. The number of experiments was necessarily limited because of the expensiveness of histamine, and it is therefore possible that an extensive series of experiments might reveal some differences in the above respect.

PEPTONE EXPERIMENTS

a. Cat's intestine

There exists a close resemblance between the action of peptone and of histamine on the isolated intestine, a similarity that might easily lead to the conclusion that the active principle of peptone is actually histamine or a substance closely related to it. Both stimulate the intestine in low or moderate concentrations and paralyze it in high concentrations. The stimulation and the inhibition look so much alike in both cases, that it would be impossible from the aspect of the curves to decide whether histamine or peptone has been used. There are certain discrepancies however in their action, which makes it doubtful that histamine is the active principle in peptone.

The minimum concentration of peptone, which had a distinct stimulating effect was found to be around 1:75,000. In some cases the stimulation by this concentration was quite apparent (fig. 14), in other cases there was no visible effect. With still lower concentrations it was not possible with any degree of certainty to get a stimulating effect. The minimum stimulating concentration of histamine lies well above 1:750,000. This

concentration practically always caused a very marked stimulation. If the two minimum concentrations are compared, it will be seen that the same or a usually more marked stimulation is obtained with histamine in a dilution a hundred times as large

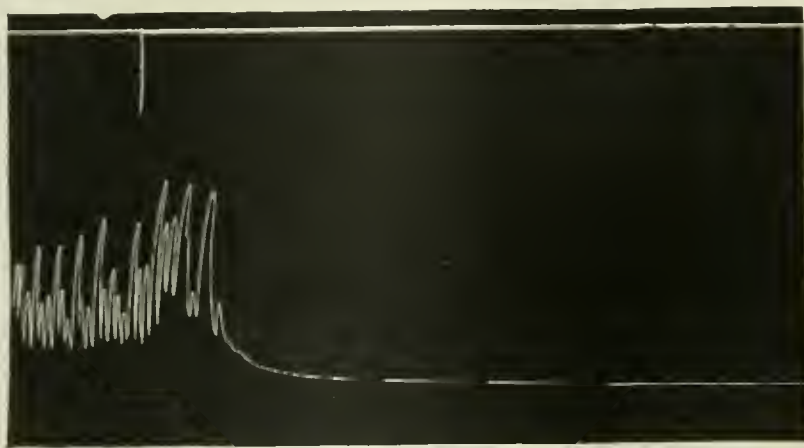


FIG. 13. SMALL INTESTINE OF CAT

Tyrode, 39°C. At *a*, peptone 1:220. The strip remained completely relaxed for about forty minutes; then slow contractions with long intervals started.

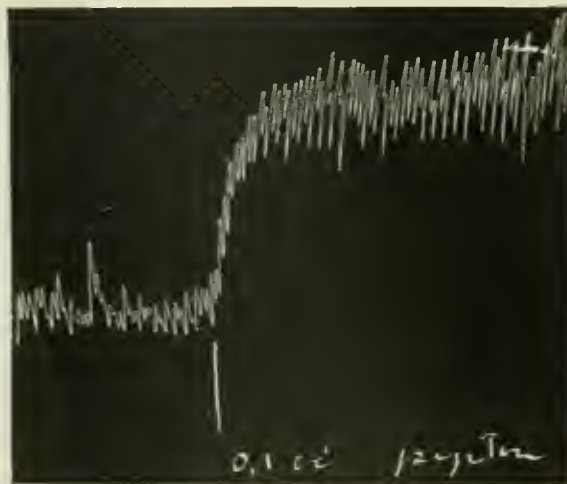


FIG. 14. SMALL INTESTINE OF CAT

Tyrode, 39°C. At *a*, peptone 1:7500. Marked increase in tonus

as with peptone. About the same difference was seen when the uterus of the guinea-pig was used as a test object. Concentrations of histamine 1:75,000,000 (fig. 15, *a*) and peptone 1:750,000 (fig. 15, *b*) both caused the same effect, a hardly notice-

able tonus increase. On the other hand the paralyzing concentrations lie much closer together. For histamine it was found to be approximately between 1:500 to 1:700. Peptone fairly constantly caused a complete inhibition in concentrations of 1:200 to 1:250 (fig. 13). The ratio between the minimum paralyzing concentrations of peptone and histamine therefore is approximately 1:3, while the ratio between the minimum stimulating concentrations is 1:100. Further when a paralyzing dose of histamine or peptone is given, the initial stimulation is usually much more marked in the case of histamine than in the case of peptone. The curves of action of the two substances



FIG. 15. UTERUS OF GUINEA-PIG

a, Ringer 39°C. At *b*, histamine 1:75,000,000. A hardly noticeable increase in tone followed. *b*, Ringer 39°C. At *a*, peptone 1:750,000. As compared with the amplitude of the contractions previous to the addition of peptone the tonus increase is about the same as in figure 15 *a* after addition of histamine.

therefore do not run parallel, and the action of peptone cannot be explained simply by the hypothesis that it contains a certain amount of histamine. Of course this does not exclude the possibility that peptone contains histamine, but in this case some of the other compound present in peptone must in some way act to modify the effect of histamine.

As in the case of histamine no complete paralysis could be elicited by injecting peptone into an isolated loop. In figure 16 can be seen that a very marked inhibitory effect resulted when 1 gram of peptone dissolved in 2 cc. of Ringer's solution was injected into a loop of approximately 10 cm. in length. In other cases this amount of peptone had only a stimulating effect.

Greater concentrations of peptone solutions were difficult to get and a larger quantity of fluid could not well be injected without involving distention of the intestine as a new factor.

b. Intestine of rabbit

The rabbit's intestine reacted to histamine mainly with inhibition. The same is the case with peptone and the inhibitory effect of peptone is even more marked than that of histamine. At the same time individual variations in the response to peptone as well as to histamine are more apparent than in the cat's intestine.

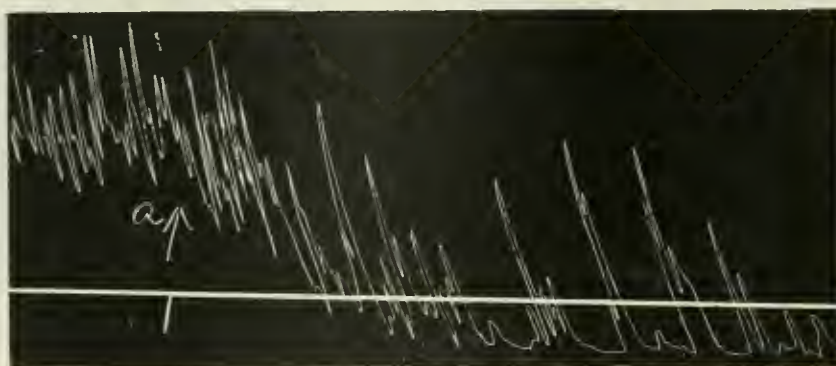


FIG. 16. ISOLATED LOOP OF SMALL INTESTINE OF CAT

Ringer, 39°C. The loop was approximately 10 cm. in length and tied off in both ends. The loop was fixed at two points by means of small hooks and with a third hook 3 cm. from one of the other connected to a muscle lever. A fine needle was inserted at one end of the loop and at *a*, 1 gram of peptone dissolved in 2 cc. of Ringer's solution was injected.

With concentrations of 1:750,000 and 1:75,000 (fig. 17) no response whatsoever was obtained. Concentrations of 1:2500 was the lowest, that with any degree of certainty gave a distinct response. Practically always this reaction consisted in a drop in general tonus. The amplitude of the contractions was sometimes also decreased but in other cases they were actually increased although the general tonus had fallen. Frequently a decrease in rate of the contractions was observed.

In experiment 20 (fig. 18) peptone in a concentration of 1:2500 caused a distinct drop in general tonus and the frequency of the beats was reduced to about one-half.

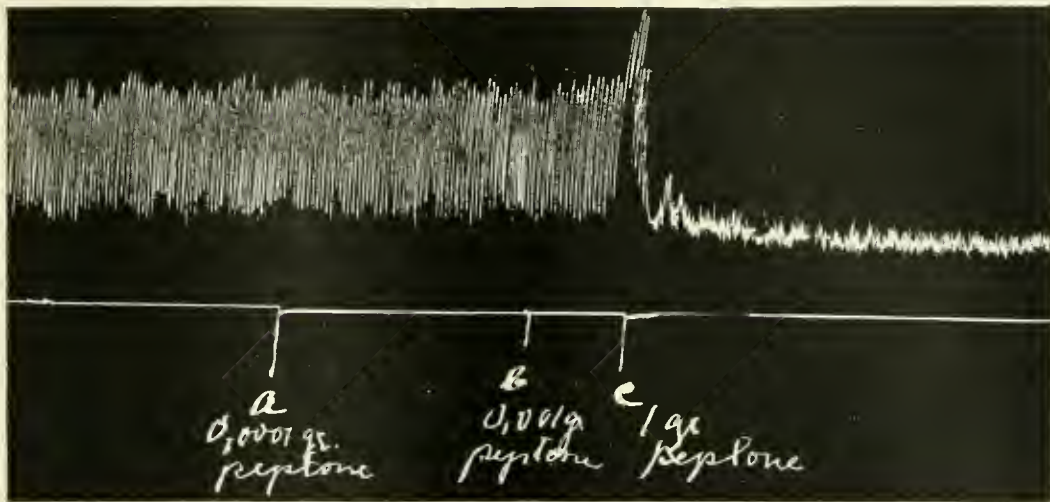


FIG. 17. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. Bath volume 75 cc. At *a*, 0.0001 gram peptone. This had no visible effect. At *b*, 0.001 gram peptone still without effect. At *c*, 1 gram peptone which after a short initial stimulation completely inhibited all contractions. The small oscillations on the curve are due to the oxygen bubbles.

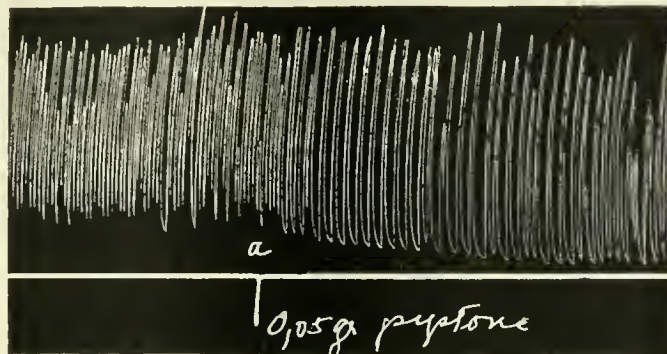


FIG. 18. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. Bath volume 75 cc. At *a*, peptone 1:2500

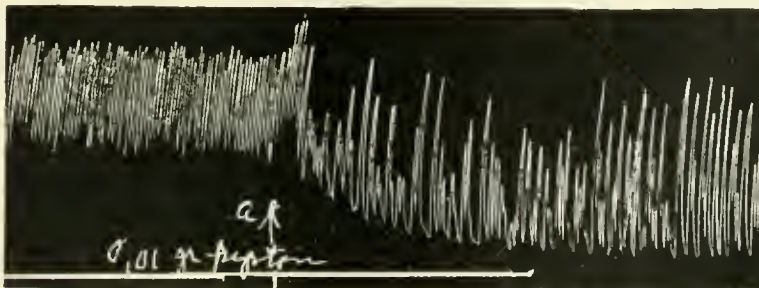


FIG. 19. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. Bath volume 75 cc. At *a*, peptone 1:750

On the other hand the amplitude of the contractions was decidedly increased. The same reaction can be seen in experiment 21 (fig. 19) where a concentration of 1:750 was used. Here the inhibition was more marked and the amplitude of the contractions were at first distinctly reduced. They soon recovered however and ten minutes after the addition of peptone the curve had very much the same appearance as in the preceding experiment (fig. 18). Another type of reaction is seen in experiment 33 (fig. 20). At *c* peptone was added to the bath so as to give a concentration of 1:750. The effect was a drop in tonus and a reduction of the amplitude of the contractions. At *d*, *e*, *f* and *g* the concentration of peptone was increased to

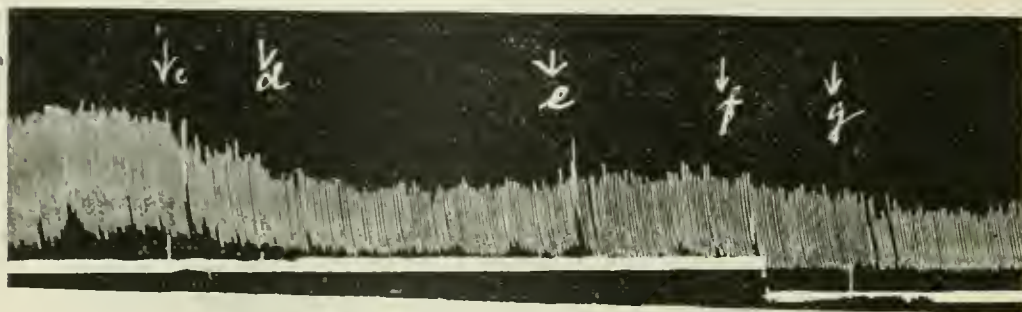


FIG. 20. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. At *c*, peptone 1:750. At *d*, the concentration of peptone was increased to 1:325, at *e*, to 1:250; at *f*, to 1:188; and at *g*, to 1:150.

respectively 1:325, 1:250, 1:188 and 1:150. Each time there was a drop in tonus and a further decrease in the amplitude of the contractions, but the reactions after the last three doses were not so apparent as after the first two.

In none of these cases could an initial stimulation preceding the drop in tonus be observed. If a still greater concentration of peptone was used an initial stimulation of very short duration could be noticed. In experiment 22 (fig. 21) a concentration of 1:85 was used. A short stimulation followed immediately on the addition of peptone, the tonus then rapidly fell and there was a complete inhibition of all contractions for about fifteen minutes. Then the intestine began to recover again, but the contraction never reached their previous rate and amplitude

and they soon began to decrease. Washing the strip failed to restore its irritability. In another experiment with the same concentration of peptone the inhibition was of longer duration and the tendency to recovery not so marked.

These experiments lead to the conclusion, that the rabbit's intestine is not quite so susceptible to peptone as the cat's. Both the minimum stimulating and minimum paralyzing concentrations are greater. The expression minimum stimulating dose is not quite correct for the rabbit's intestine, as the effect of even very high dilutions was mainly inhibitory. The expression may therefore serve to indicate the smallest concentration, which had any effect at all.

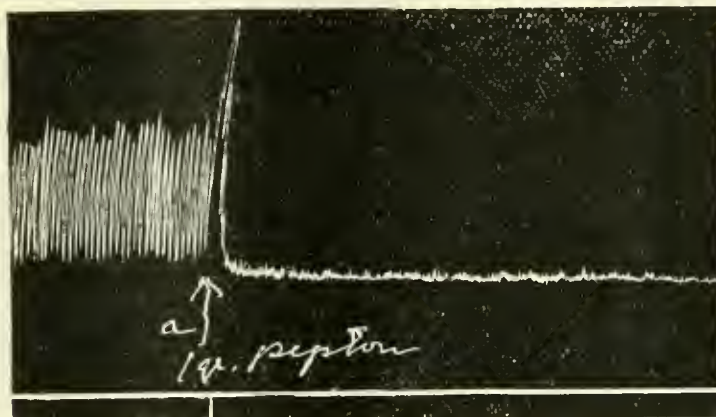


FIG. 21. SMALL INTESTINE OF RABBIT

Tyrode, 39°C. At *a*, peptone 1:88

When tested on the rabbit's intestine the curves of action of histamine and peptone run more closely parallel than in the case of the cat's. The minimum concentrations, which had any appreciable effect upon the rabbit's intestine were for histamine 1:75,000 and for peptone 1:2500. To obtain complete inhibition concentrations of 1:700 and 1:85 were necessary. Obviously there still is a discrepancy although not so large as in the cat's intestine.

A point of interest emerging from this investigation is the relation between the paralyzing concentrations of histamine and peptone. As already mentioned Dale and Laidlaw (14) have suggested that the pharmacological activity of peptone

depends upon the presence of histamine or some substance related to it. This explanation does not seem to hold good with regard to the inhibitory action of peptone on the isolated intestine. Under the assumption that histamine accounts for the stimulating effect of peptone on the smooth muscle of the intestine, the peptone used in these experiments would contain approximately $\frac{1}{100}$ of its weight of histamine. This approximation is deduced from the fact that the same stimulating effect on the cat's isolated intestine is obtained by solutions of histamine and peptone of 1:750,000 and 1:7500. The same tissue was paralyzed by a concentration of histamine of 1:500 to 1:700. If histamine were solely responsible for the inhibitory action of peptone in large concentrations, the necessary concentration of peptone to bring about complete inhibition would be 1:5 to 1:7. Now the inhibition actually occurred at peptone concentrations of 1:200 to 1:250. Still under the presumption that our peptone contains 1 per cent of histamine, the calculated concentration of histamine in a peptone solution of 1:250 would be 1:25,000. In this concentration however histamine has a purely stimulating effect on the cat's intestine, and the inhibition therefore can hardly be attributed to histamine.

The figures quoted above referring to the minimal concentrations of histamine and peptone still having a distinct stimulating effect on the cat's intestine, and the amount of histamine calculated from these figures as present in peptone can not claim to be more than approximate. Only a large number of experiments could completely eliminate the influence of the individual responsiveness of different preparations to histamine and peptone. The variations found in these experiments however all point in the direction that the discrepancies in the stimulating power of histamine and peptone are still greater than is indicated by the above figures, which thus represent the minimum of difference found in a considerable number of experiments.

It was rather surprising to find that histamine has a comparatively weak stimulating effect on the rabbit's intestine. In almost every case the main effect was an inhibition, and when a stimulation was observed it was in no case at all comparable

with the very powerful stimulation of the cat's intestine. The individual variability of the response also was much more pronounced than in the case of the cat's intestine. The same concentration of histamine would in one case cause a stimulation, in another inhibition and in a third no effect at all.

The only case where a stimulation was seen with any degree of constancy was when strong concentrations of histamine were used. In these cases there was always an initial stimulation followed by an inhibition, but the stimulation was quite insignificant compared with what was seen in the cat under similar circumstances. If this preponderance of the inhibitory action of histamine on the rabbit's intestine was dependent upon a greater sensitiveness to histamine and consequently of a displacement of the inhibition threshold towards lower concentrations of histamine, one would expect that a complete inhibition could be obtained with a lower concentration of histamine than was the case with the cat's intestine. This does not seem to be true however; at least the difference between the paralyzing concentrations is not greater than can easily be explained by individual variations. This relative insensitiveness of the rabbit's intestine to histamine corresponds with the difference in action of histamine when injected intravenously in a cat and in a rabbit.

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Samuel James Meltzer

The death of Dr. Samuel James Meltzer on November 7, 1920, closed the career of one of the most eminent workers of our day in the fields of physiology and pharmacology. Meltzer was born in Curland, Northwestern Russia, on March 22, 1851. Destined by his parents for the rabbinical career, the talented youth, after a long internal struggle, decided to devote his powers to a field of secular learning. He was married at the age of nineteen, moved to Königsberg and attempted to learn a trade in order to support himself and his family. This attempt resulted in failure but he nevertheless remained in Königsberg and there prepared himself to enter the University of Berlin. In 1873 he was graduated from the Real Gymnasium at Königsberg and in 1876 he entered the University of Berlin at first as a student of philosophy but he soon turned his attention to medicine. He remained at the University from 1876 to 1882 in which year he received the degree of M.D. In 1883 he came to the United States paying his way by performing the duties of a ship's surgeon on the vessel that carried him over. He began at once to practice medicine in New York City and with the financial assistance of friends was soon able to have his family join him. The imperative necessity of providing for his family made it impossible for him to carry on research work immediately after coming to this country, but when he was only partly on his feet he began to work in Welch's laboratory of pathology at the Bellevue Hospital in the fall of 1883. Later he attached himself to the physiological laboratory of Curtis in Columbia University and then to the pathological institute of Prudden. In 1904, he relinquished his large medical practice and became as associate of The Rockefeller Institute. In 1907 he was honored by being made the head of its department of physiology and pharmacology.

Meltzer began his career as an investigator by working with Kroecker in 1880 and subsequently on the "*Schluckmechanismus*" and its nervous control. The two authors published valuable researches on this subject in 1881 and 1882, and in the years immediately following Meltzer occupied himself with the analysis of the rôle of the central nervous system in the act of swallowing. His lifelong interest in the mutual play of excitation and inhibition in physiological processes dates from this early work.

In 1884 appeared the results of his first researches in America, a joint paper with Welch—an examination into the behavior of red corpuscles when shaken with various substances. The ideas here outlined were later (1892 and 1894) more fully developed in papers entitled, "On the Importance of Vibration to Cell Life." In the decade 1884–1894 he published numerous papers on pathological and clinical as well as on physiological subjects. The relationship between inhibition and excitation in swallowing and in the respiratory act continued to occupy his thoughts. He gave expression to his ideas in this field of physiology by stating that the relationship between certain functioning muscle groups may be expressed in terms of a law of *Reciprocal Innervation*.

or *Antagonistic Innervation*. Here is a field in which Meltzer undoubtedly did valuable work as a pioneer.

In the following decade came researches on the faradization of the alimentary canal in animals, on the paths by which fluids are carried from the peritoneal cavity, on the non-absorption of strychnine by the gastric mucous membrane, on peristaltic movements in the oesophagus, on the methods and means employed by the animal organism in its struggle against bacteria and on many more subjects. During this period there also appeared joint researches with his daughter Clara on the vasomotor nerves of the rabbit's ear, on the influence of suprarenal extracts on the blood vessels and on other problems connected with the section of sympathetic nerves.

The next decade, 1904 to 1914, was a very fruitful period for Meltzer and his pupils as it was in this decade that his connection with the Rockefeller Institute first gave him full opportunity for the display of his talents. In 1904 he discovers with his daughter the paradoxical pupil-dilatation caused by adrenalin. In 1905 he describes with his son-in-law, John Auer, his remarkable discoveries in relation to the pharmacological properties of the salts of magnesium and the dramatically curative and antagonistic action of calcium salts in the complete unconsciousness and muscular paralysis induced by salts of magnesium in the rabbit. Numerous important papers now appeared, written in conjunction with Auer, Joseph and Lucas which deal with the pharmacological properties and clinical uses of salts of magnesium, calcium, strontium and sodium. Other researches of this decade deal with the sensibility of the abdominal organs, the late effects of the removal of the superior cervical ganglion; "peristaltic rush;" the direct and indirect irritability of the muscles of frogs; experimental pneumonia produced by intrabronchial insufflation; intratracheal and pharyngeal insufflation and anaesthetization by intratracheal insufflation.

Meltzer's extensive researches on artificial respiration through intratracheal and pharyngeal insufflation naturally gave him a leading place on three national commissions on resuscitation. He devised a simple, portable apparatus for resuscitation by pharyngeal insufflation. Other researches of this decade and of the years following 1914 show that neither advancing years nor ill health could weaken his dauntless spirit. The old subjects as well as newer ones like shock, continued to occupy his alert mind. In his last paper which appeared in the September number of the Proceedings of the National Academy of Sciences, he announced the conclusion that the superior cervical ganglia contain a principle which is essential to the maintenance of life.

During his long and active career Meltzer also wrote many papers of general interest and not the least of his services in writing these essays was that of bringing about a better mutual understanding between laboratory workers and medical and surgical practitioners. Only a well endowed man, imbued with high ideals, not given to self-seeking, possessed of great tenacity of purpose and the ability to toil unremittingly could hope to attain the position or exert the influence which were Meltzer's at the time of his death. Not America alone but all countries are in need of more men of his type. Those who during many years have had frequent contact with this extraordinary personality, still more those who were privileged to call him an intimate friend, generous and stimulating, will deeply mourn his passing.—J. J. A.

STUDIES ON THE INFLUENCE OF SEDATIVES ON ANIMALS. I

A METHOD FOR MEASURING THE INFLUENCE OF STIMULATING DRUGS AND OF SEDATIVES ON THE ACTIVITY OF ANIMALS (ACTIVITYMETER)

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During the last years I have been engaged in studying the quantitative relations between the concentration and the action of drugs. I found (1) that it was possible from this point of view to arrange a number of drugs into two different groups. First, those narcotics which are easily soluble in lipoids and second, the alkaloids and other basic poisons. In the course of these experiments it was found useful to make graphic records for the relation between the concentration and action of the drugs. We plotted on the abscissa the concentration in which the drugs acted on isolated organs or the doses of the drug per kilogram of animal and on the ordinate the action of the drug, viz., height of blood pressure change, contraction of isolated organs, increase or decrease of reflexes in decerebrated animals, etc. A graph constructed in this way we called a concentration effect curve. If such curves are made one finds that the drugs belonging to the first of the groups mentioned above (ether, chloroform, chloralhydrate, urethane and also magnesium sulfate) all give curves resembling that shown in figure 1, whereas the alkaloids and other basic poisons give curves resembling rather closely the type presented in figure 2. The relation between the concentration and the effect in figure 1 is easy to understand. When the dose of the drug is doubled the action is also increased in the same rate. In the second group however

the relation is quite different. There is in this case always a zone where a small increase in the dose produces a large increase in the action. The character of the curve then changes to a

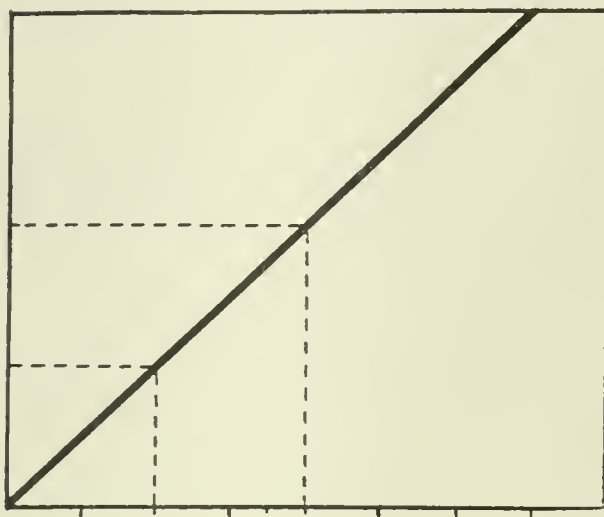


FIG. 1. CONCENTRATION-ACTION CURVE TYPE I

Ordinate: action of drug. Abscissus: concentration of drug [doses per kgm. animal].

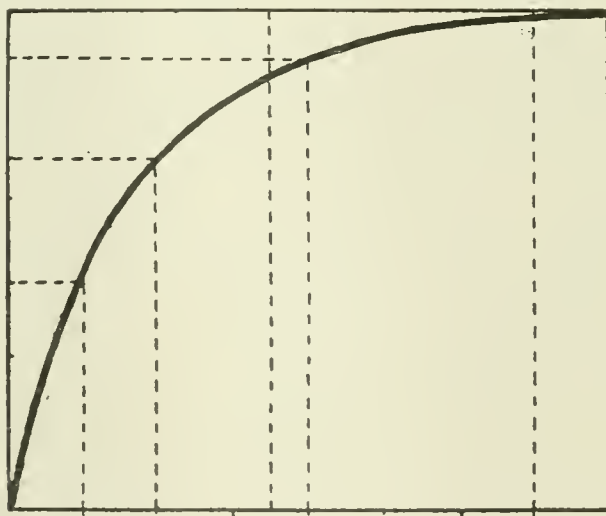


FIG. 2. CONCENTRATION-ACTION CURVE TYPE II

phase wherein a large increase in the dose results in only a slight increase in action.

In the experiments referred to above we had already studied the concentration-effect curve of a number of narcotics and other poisons like adrenaline, pituitrine, nicotine, lobeline, etc.

We were, however, of the opinion that it might be very useful to extend these researches to another group of poisons, viz., the sedatives. From many of the sedative agents, veronal, sulfonal, chloral, adaline, etc., numerous things relating to their chemical or pharmacological properties have been experimentally studied during recent years. But that property of these drugs which would interest us most, viz., the sleep-promoting or sleep-inducing actions has never been quantitatively determined.

Using the terminology which has been proposed above one can say, that the concentration-action curve of these drugs is fully unknown. The minimum effective dose of these drugs that can promote or induce sleep in an animal or in man has not yet been determined nor has been studied in what degree this sleep promoting power is intensified if the dose of the drug is doubled or tripled. It has been impossible to study these things because there was no method to give exact quantitative information for the sleep promoting power of these drugs.

It goes without saying that it would be very difficult to find a method which would really allow to measure sleep in animals and to investigate the influence of drugs thereupon. The author hoped however that it would be possible to measure a factor that stands in very close relation to sleep, viz., the quantity of movements made by an animal during night. If a quantitative measurement of the movements or of the "activity" of a dog during night could be made, it would very probably be possible to make a quantitative study of the action of sedatives and other drugs on the activity of the animal and it was esteemed, that roughly spoken, a decrease of the activity of the animal could be considered as an indication of a more profound or more prolonged sleep, whereas an increase of the movements during night would indicate a shorter or a less profound sleep.

In order to be able to investigate the matter of the influence of sedatives on animals, it was deemed necessary therefore to have at one's disposal an apparatus which would enable us to measure the amount of movements made by an animal during a period of several hours.

Talking with Dr. Benedict in Boston about the difficulties that exist in this field he gave a very useful suggestion which enabled me to have constructed an apparatus, that—although being far from ideal—opens the possibility to study quantitatively the relationship between the dose and the action of seda-

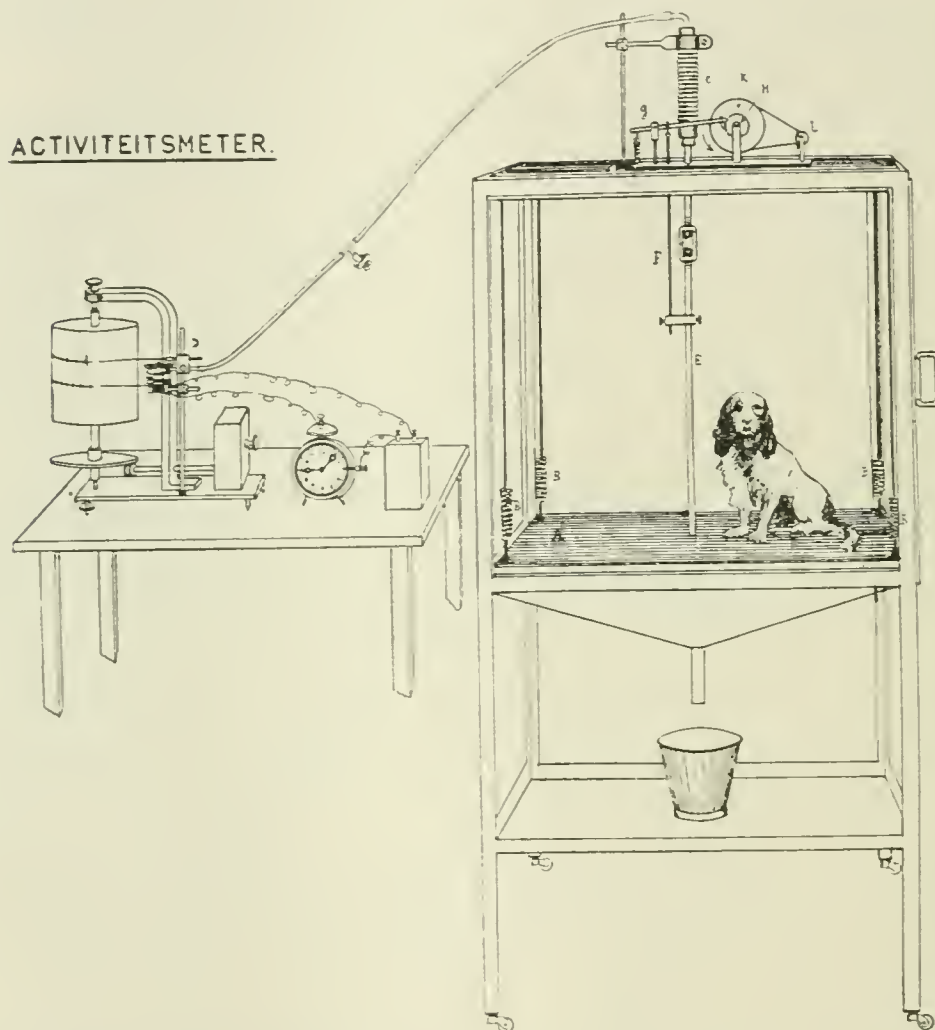


FIG. 3. APPARATUS FOR MEASURING THE INFLUENCE OF DRUGS ON THE ACTIVITY OF ANIMALS (ACTIVITYMETER)

tives. Dr. Benedict kindly demonstrated a device whereby he records the movements of animals which are enclosed in his respiration chambers. By using this principle—which in fact is a modification of a device described first by Czymanski—I constructed the apparatus shown in figure 3.

This arrangement consists of an ordinary cage (Hans Meyer cage) the bottom of which, *A*, is suspended on four coil springs, *B*, so that the movements of the animal—till now always dogs were used—set it in motion in a perpendicular direction. These motions of the cage are transmitted by an adjustable rod, *E*, to a pneumograph which consists mainly of a piece of corrugated rubber tubing (*C* in fig. 3) and are registered by means of the usual Marey tambour, *D*, on the drum of a slowly moving kymograph. Also the movements of the bottom of the cage operate a lever, *G*, one end of which carries a pawl that engages with a fine toothed wheel, *H*. By means of a pulley, *K*, a Veeder counter, *L*, is made to record the number of revolutions. In this way it is possible to procure a quantitative statement for the movements, made by an animal occupying the cage during a certain period. (The graphic record provides for analysis as to distribution, size, etc., of movements.)

It is then possible to get an insight in the amount of movements made during a certain period by an animal placed in the cage. We found it useful to put the cage in a quiet room in the laboratory and always started our experiments at 6.00 p.m. The kymograph was set in motion then, the number on the Veeder counter was read and care was taken, that nobody entered the room till 8.00 a.m. One of the curves gained during a period from 5.00 p.m. till 8.00 a.m. is represented in figure 4. In this figure the upper line indicates the movements of the dog, the lower line gives the time in hours. The time was registered by means of an ordinary alarm clock with electrical connection. In the first experiments we always set the alarm at 2.00 a.m. hoping that the animal would be waked up by this noise, it seemed however that an alarm clock is not an adequate stimulus for a dog as we never registered an unusual number of movements at 2.00 a.m. The movements made by the dog between 5.00 p.m. and 6.00 p.m. are not to be taken into consideration as they are due to accessory stimuli, i.e., entering of servants in the room, setting the kymograph in motion, etc.

In figure 5 a curve is represented of the same animal that was used in figure 4, but now this animal (a dog of 7 kgm.) had at



FIG. 4. MOVEMENTS OF DOG DURING NIGHT

Upper curve; tracing of activitymeter. Lower curve; time in hours. Note the increase in activity of the dog between 5.00 p.m. and 6.00 p.m. caused by entrance of servants in the room.

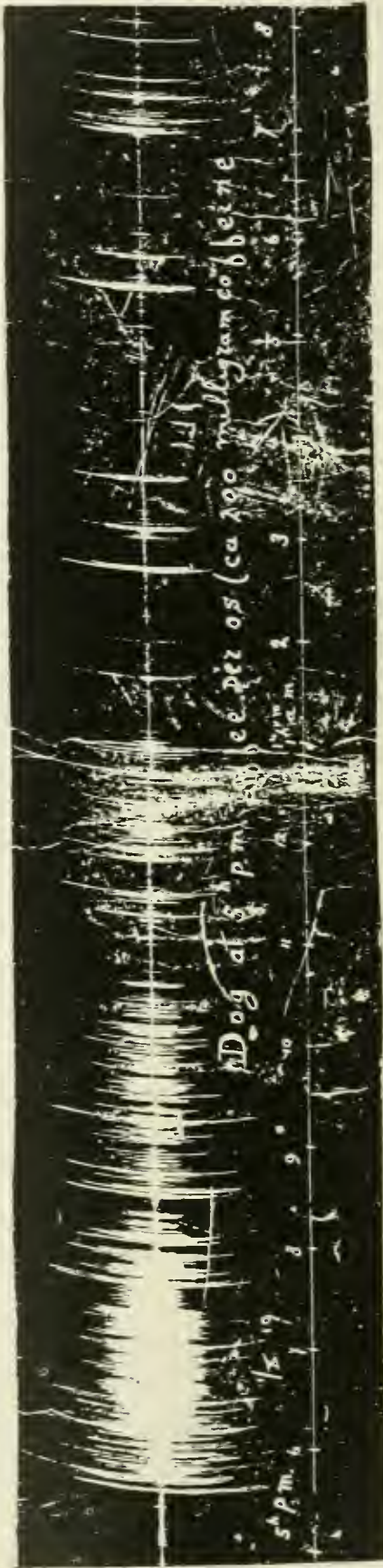


FIG. 5. MOVEMENTS OF DOG DURING NIGHT AFTER A LARGE DOSE OF COFFEE (CONTAINING ABOUT 200 MGM. CAFFEINE) PER OS

5.00 p.m. received a large dose of coffee per os containing about 200 mgm. gram of coffein at 5.00 p.m. The curve gives rather a good impression of the influence of this dose of coffein on the movements of the animal, but of course it is not possible in this way to make quantitative determinations. This however can be done by reading the number on the Veeder counter before and after the experiment. In one series of experiments we found that during seven consecutive days the average number of revolutions recorded by the counter between 6.00 p.m. and 8.00 a.m. was 36, whereas after the injection of a large dose of coffein it was 248.

It goes without saying that this method permits also to study the influence of sedatives on the mobility of the animal. In how far this can be considered as a study of the sleep inducing power of these drugs has been discussed already above.

In using the described apparatus for the study of the influence of sedatives one can proceed in two ways. In the first place one can observe the effect of a sedative after having taken the normal readings of the animal during a couple of days. This has the disadvantage that with many animals the number of revolutions recorded during one night is very low so that one cannot expect to get very definite changes after having given a sedative. Often therefore it will be better to follow another line, viz., to study first the effect of an exciting drug, i.e., coffein and then trying to find a dose of the sedative that just cuts down the increased movements of the animal to normal again. Both ways are followed in a series of experiments on dogs and monkeys which are in proceeding now in this laboratory and the results of which will be published later.

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ON THE DURATION OF CONSTRICTION OF BLOOD-VESSELS BY EPINEPHRIN¹

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INTRODUCTION

In the history of physiology of bloodpressure the discovery of vasoconstrictor and vasodilator nerves in the fifties of the last century by the classic physiologists Claude Bernard, Brown-Sequard and Schiff, marked an important event. It was made by simply observing the behavior of the blood vessels in the ears of rabbits after section of the sympathetic nerve, and after stimulating the central end of the sectioned nerve. In the desire to obtain quantitative data and preservable illustrative records, bloodpressure was studied in physiology in the subsequent years chiefly by the graphic method. When later another important event in the physiology of bloodpressure was brought to light, namely, the action of the suprarenal extract upon the blood-vessels, it was studied nearly exclusively by the graphic method. It is the method of choice to this day. It is true that Oliver and Schafer (1) in their elaborate paper state that "direct ocular

¹The nomenclature in the literature on adrenal extract is confusing. The following explanation of the terms we use in this paper may not be amiss. Three conditions are to be distinguished. 1. The secretion of the adrenal glands, or its principle as it exists in the living body, or is supposed to exist there. For this we accept the term Adrenin, suggested by Schafer and being used by various authors abroad and in this country. 2. The active principle as isolated outside of the body (adrenalin, suprarenin, etc.). Abel was the first to isolate the principle in the form of a mono-benzoyl derivative and named it epinephrin. The American Pharmacopeia made this designation official. We shall use it as a generic name. 3. The particular preparation which is used in a specific investigation. In this series of experiments the adrenalin solution of Parke, Davis and Company was used.

evidence upon both the large and the small bloodvessels" was one of the methods employed in their studies. This was apparently done by simply looking at the bloodvessels in the tissues after a surgical exposure. But the exposure of normally protected bloodvessels to atmospheric air is a circumstance which can not well be disregarded when estimating ephemeral changes in the size of these vessels.

As far as we know, S. J. and C. Meltzer were the first to study the action of epinephrin upon bloodvessels by ocular observation of the rabbits' ear. While trying to establish the share which the sympathetic and the large auricular nerves possess in the innervation of the ear vessels (2) they discovered that the reaction of these vessels to an intravenous injection of adrenalin differs in ears deprived of their vasomotor innervation, from that of normal ears in several respects: the latent period is longer, the development to the maximum and the decrease to normal is slower, and the entire duration of blanching of the denervated part is much longer than that of the normal ear (3). Later these authors administered adrenalin to rabbits by subcutaneous injection in some part of the back. They found that large doses which are capable of causing a prostration of the normal animal, produce a blanching of the ears. However, subcutaneous administration of medium or rather small doses, while affecting the denervated ear in the same direction as an intravenous injection, causes rather a dilatation of the vessels of the normal ear (4). Finally in studying the influence of the sympathetic nerve and the superior cervical ganglion upon inflammation in the ears of rabbits, these authors (5), in order to bring out certain differences, injected adrenalin subcutaneously in the ear itself; it caused a contraction of the dilated vessels in the "inflammatory area" (Entzündungshof) but did not affect the vessels in the "inflammatory focus" (Entzündungsherd).

The study of the action of epinephrin upon bloodvessels in the rabbits' ear is in many respects an advantageous method. The animal remains in a normal state; it is not exposed to the influence of an anesthetic and need not undergo preliminary

operations. No apparatus is required for the observation. By this method medium-sized arteries and arterioles, veins and even capillaries (paleness and flushing of the areas between visible vessels) are studied with ease. By this method the bloodvessels are studied directly and not the average bloodpressure of the entire animal; they are studied in an organ, the ear, normally connected with the circulation of a living animal and not in a perfused organ, which is provided only with nervous and circulatory mechanisms and not, in addition, with mechanisms for chemical activities which might modify the action of adrenin upon the bloodvessels.

In two studies the present writers (6) established the unmistakable difference in the results of intramuscular and subcutaneous injections. The effect of an intramuscular injection sets in practically as rapidly as after an intravenous injection, while the effect of an subcutaneous injection upon bloodpressure develops slowly and is comparatively insignificant. In giving a subcutaneous injection, great care must be taken that the needle does not enter muscles or pass beneath a muscle fascia; such a mishap is not likely to occur when subcutaneous injections are given in the ear; the muscles of that structure are small and thin, and it is hardly possible to make there an "intramuscular" injection.

EXPERIMENTAL.

During the last few years we carried out experiments at various times in which adrenalin was injected subcutaneously in one ear of a rabbit and the course of the reaction of the various bloodvessels was studied by ocular observation. We wish to report here the results obtained in these experiments. In the following account, one or two protocols will be given to illustrate the several groups of experiments. Many of these accounts will be very brief and will consist essentially in a summary.

In the present series of experiments mostly the commercial solution of adrenalin chlorid of Parke, Davis and Company (1:1000) was used. In a few instances the solution was warmed for the purpose of removing the chloretoe. It was shown later however, that the warming could

be dispensed with. Injections of chloretone, or of chloretone with HCl of an acidity equal to that of the adrenalin chlorid, had no effect upon the conditions of the bloodvessels. In a few experiments the solution was freshly prepared in the laboratory from adrenalin crystals.

In the first few experiments the injection of adrenalin was made subcutaneously on the external surface of the tip of the right ear near the central artery and below its inner branch.

Experiment 1. White female rabbit, 1440 grams. 2.35 p.m. Injected 0.3 cc. adrenalin; lost one drop. Observed until 5.05 when the right ear was still pale. Next day the right ear flushed definitely more than the left.

Summary. There was a strong constriction of practically all the bloodvessels of the injected ear. There was no flushing phase after handling of the animal. The pallor was uniform and lasted about three hours; (no observations made after this time). Only the outer lower third (external to the central artery) showed one hundred minutes after the injection a slight dilatation of the vessels. The left, uninjected ear became engorged shortly after the injection into the other ear. But after five minutes the ear vessels of the left ear showed quite rapid fluctuations. Next day the right, injected, ear gorged more readily than the left, and stimuli which caused blanching of the left ear did not affect the engorgement of the right ear.

Experiment 2. White female rabbit, 2330 grams. 11.52 a.m. Injected 0.3 cc. adrenalin; (about five drops lost). Observed until 5.05 when injected ear was still fairly pale.

Summary. In this experiment 0.3 cc. was injected and about five drops were lost, that means that actually only a very small amount of adrenalin was injected. Nevertheless there was no dilatation of the vessels in the injected ear. On the contrary, the constricting effect set in immediately after injection and lasted several hours. The intensity of the constriction however was less than in the foregoing experiment. The uninjected ear paled for a very brief time and was followed by an engorgement.

The two foregoing experiments showed unmistakably that an injection of a small quantity of an adrenalin solution in the tip of the ear causes at once a paleness of practically the entire ear which lasts for several hours. The question arose: is the effect due to adrenalin? In the tip of the ear the connective tissue is rather dense. A subcutaneous injection of 0.3 cc. of any solu-

tion will cause in this place considerable pressure. Could not the observed effect be due to the mechanical factor of pressure and not to the chemical element of epinephrin? This point was tested in experiments with saline of which the following is an example.

Experiment 3. White female rabbit, 1500 grams. 10.17 a.m. Injected 0.3 sterile 0.9 per cent NaCl subcutaneously into the tip of the right ear.

Summary. After an injection of 0.3 cc. of 0.9 per cent NaCl into the tip, two thirds of that ear became pale at once and the central artery became small. However, the paleness began to recede eight minutes later, the middle third being already pink and the central artery beginning to get wider. Twenty-three minutes after injection there was paleness only in the upper fifth; in the other parts this ear resembled the non-injected ear. Immediately after the injection a ridge appeared along the central artery extending to the lower third. That ridge had largely disappeared in less than thirty minutes. It seemed to have been formed by the presence of fluid within the sheath of the artery. The injection in the one ear did not seem to have any effect upon the other ear.

Experiment 3 shows that an injection of normal saline into the tip of the ear causes indeed paleness of a large part of the ear and the central artery becomes narrow. The paleness however begins to disappear in less than ten minutes and is practically gone in less than thirty minutes. In the two experiments in which adrenalin was injected, the paleness persisted uniformly for more than one hour and a half and remained greatly in evidence for more than two hours, or rather as long as it was observed. While we have to admit that the pressure caused by the injection of adrenalin may be an important element in the early and sudden development of the blanching of the ear, there can be no doubt that it is the chemical property of the injected solution which is largely responsible for the long duration of the blanching.

In order to eliminate the mechanical factor as much as possible, the injections were made henceforth in the base of the external surface of the ear, where the subcutaneous tissue is more

loose than in any other part of the ear. The pressure caused by the subcutaneous injection of fluid in this place could hardly drive it upwards mechanically; it is more liable to force the fluid into the loose tissues of the neck and to the tissues of the base of the other ear. The needle was introduced just above the transverse vein, pushed downward under the vein and the fluid injected below it.

Experiment 4. White female rabbit, 1740 grams. 2.48 p.m. Injected 0.5 cc. adrenalin subcutaneously into the base of the right ear, externally to the central artery and about midway between it and the outer margin of the ear. Observed until 5.05 the same day and the next day after 10.30 a.m.

Summary. The injection of 0.5 cc. of adrenalin into the base of the ear caused paleness of the injected ear. But the intensity of the pallor was not as great as after an injection in the tip. The pallor did not set in at once; it developed gradually. The injection was made externally to the central artery and the pallor was greater in this part. (The central vessels adhere to the skin and form a sort of a partition between the outer covering and the cartilage of the ear.) The paleness lasted for nearly three hours, that is, as long as it was observed. Next day it was the injected ear which showed a distinct tendency to dilatation.

The non-injected ear showed promptly a tremendous dilatation shortly after injection.

Experiment 5. (Full protocol.) White female rabbit, 1760 grams. 11.08 a.m. Injected 0.5 cc. adrenalin subcutaneously into the base of the left ear; 0.25 cc. on both sides of the central artery, near the artery.

11.08½ Left ear pale, right more full; marked difference; right ear fluctuates; left pale from the base to the tip.

11.10 Very marked difference; left pale, no blood in central artery and upper branches. Right fills up and then pales on excitation.

11.30 Left very pale; artery not visible; veins very small and narrow. Right dilates only moderately.

12.15 Left very pale; artery practically not visible; veins very small; ear slightly bluish. Right fills up only moderately.

12.33, 2.00 and 3.00 p.m. Same as at 12.15 p.m.

4.25 Left central artery shows as a thin line now; left ear still considerably paler than right.

Next day 10.30 a.m. Both pale at first, then left flushes well, followed by right; both finally equal (left perhaps a bit wider).

Summary. In experiment 5, 0.5 cc. adrenalin was injected near the artery, 0.25 cc. on each side. In thirty seconds the left ear became pale from base to tip and soon there was no blood in central artery and its branches. This extreme blanching maintained for four hours, and even after nearly five and a half hours the constricting effect was still in evidence. Next day there was some tendency of the vessels of the injected ear to dilatation.

Experiment 6. (Full protocol.) White male rabbit, 1600 grams. 1.33 p.m. Injected 0.3 cc. adrenalin each side of the central artery of the left ear, away from central artery, near outer and inner margins. For twenty minutes no effect can be seen; left ear cooler than right; then smaller arteries of the left ear paled.

2.20 Now left central artery moderately contracted, left ear cooler.

2.40 Left central artery now entirely empty.

2.50 Left central artery relaxed moderately.

3.05 Left ear pale, right moderately gorged; left central artery shows occasionally small sections of blood passing slowly upwards.

4.50 Left ear pale, central artery not visible; right ear after excitation now as pale as left. But shortly right ear gorges while left remains pale.

Next day 10 a.m. Both pale at first, left perhaps a trifle less than right. After some time both filled up strongly and equally, later maximal engorgement, equal.

Summary. In experiment 6 the injections were made, 0.3 cc., near each margin, away from the central artery. For twenty minutes there was no visible effect although the ear began to be cooler; the smaller arteries began to constrict at least twenty minutes before the central artery was seen to be moderately contracted. Gradually the blanching became well pronounced and lasted for about three hours after the injection. Spreading from the tissue of the margin seems to be a slow process and it appeared to reach some distant small vessels before it reaches the central artery.

In the above experiments with injection of adrenalin into the base of the ear there was a definite, long lasting blanching of the ear and a constriction of the central artery. In experiments in which the injected adrenalin came immediately in contact

with the central artery, the blanching set in practically at once which seemed to be initiated by the constriction of the central artery. When the adrenalin was injected about midway between the margin and the central artery, the blanching and the constriction of the vessels developed gradually, and in the experiment in which 0.3 cc. of the adrenalin was given near the ear margins, the blanching began to make its appearance about twenty minutes after the injection, although the lower temperature of the injected ear was manifest before. The constriction of the central artery became evident much later than that of the smaller vessels. In outspoken cases of blanching there was no doubt that the veins participated in the constriction; but now and then some veins contained dark blood. When the constriction was not maximum, the central artery often had a moniliform appearance and short narrow sections were seen to be traveling in a peripheral direction. The constricting effect lasted in some instances as long as six hours, and certainly never less than three hours. In most of the experiments, even with intact innervation, a tendency to dilatation in the injected ear was manifest the day after the injection. In several experiments the blanching of the injected ear was accompanied by an engorgement of the other ear, the flushing setting in shortly after the injection.

Considering the readiness with which fluid injected into the loose tissue of the base of the ear makes its escape into the loose tissue of the neck, a quantity of 0.5 cc. may amount to a very small dose, especially when compared with a dose of 0.3 cc. injected into the very dense tissue of the tip of the ear. In the following experiments larger quantities were tested.

Experiment 7. White female rabbit, 1920 grams. 10.59 a.m. Injected 1 cc. adrenalin subcutaneously into the base of the left ear below the transverse vein, midway between the central artery and the external margin of the ear.

11.03 No definite difference between ears.

11.10 Both fairly pale on handling; then right ear fills up quite promptly, while left ear vessels dilate only very slightly. Observed until 4.27 p.m. the same day and again at 10 a.m. the next day.

Summary. In experiment 7, 1 cc. of adrenalin caused a markedly greater effect than 0.5 cc. when injected in a similar manner. The development was slow, but the constriction and paleness attained a marked degree and lasted for more than five hours. There was only a suggestion of a dilating effect upon the other ear and there were no definite after-effects the day following the injection.

Experiment 8. (Full protocol.) White female rabbit, 1600 grams. 11.34 a.m. Injected 0.8 cc. adrenalin into the subcutaneous tissue at the base of the left ear, 0.4 cc. each side of the central artery, and near it.

11.35 Both ears flush strongly, then left central artery begins to pale from below upward until only a thin red line remains; rest of left ear (veins) well distended. Right ear flushed; central artery wide.

11.36 Left ear pale, veins emptied; right slightly pale but central artery well dilated.

11.40 Left pale, only little blood in veins; artery a thin red line. Right shows a fairly dilated central artery, patches of right ear pale; large veins pale. Does not flush as strongly as before; but strong difference between ears.

11.57 Left central artery relaxes slightly occasionally; but ear does not fill up like right.

1.30 p.m. Left very pale, central artery just visible; slight fluctuation but never flushes; veins narrow; right flushes maximally after short period of blanching on excitation.

3.20 Left pale; right gorged maximally; striking difference.

4.00 Left central artery a mere thin line up to bifurcation at tip; beyond that moderately full; vein in upper inner part of outer surface fairly full, much fuller than before; left ear very pale; right ear usually gorged.

5.10 Left pale; central artery fairly visible; lateral veins not as prominent as before. Right pales on excitation but swiftly fills up strongly, central artery fairly wide.

7.40 Left central artery now widens slightly; right ear flushes strongly and central artery dilates well; still good difference between ears.

10.35 Left ear now flushes maximally; after excitation both pale and then left begins to dilate promptly before right and reaches maximal engorgement before right. Right finally flushes just as much as left.

Summary. In this experiment 0.4 cc. of adrenalin was injected on each side of the central artery and near it. Shortly after the injection

the entire artery began to constrict from below upward, the veins empty their contents a minute later. Eight hours after the injection there was still definite evidence of constriction. But after twelve hours the injected ear showed a tendency to flushing. The non-injected ear seemed to have reacted with a tendency to flush after the injection of adrenalin into the other ear.

Experiment 9. White female rabbit, 2570 grams. 10.42 a.m. Injected 1 cc. 0.9 per cent NaCl into base of the right ear; injected fluid spreads to central artery. Observed until 4.18 p.m. the same day and at 10.30 a.m. the next day.

Summary. One cc. of NaCl 0.9 per cent injected into the base of the ear had no effect upon the width of the bloodvessels of the ear, although the mass of the injected fluid reached the central artery.

Experiment 10. (Full protocol.) Consists of observations made simultaneously on two rabbits. Rabbit A, white, 1500 grams and rabbit B, white, 1170. Each rabbit was given an injection of 1 cc. of a very dilute adrenalin solution into the base of the ear, 0.5 cc. on each side of the central artery and 5 mm. away from it. The solution was made up of 1 cc. adrenalin diluted to 100 cc. with 0.9 per cent NaCl equalling a solution of 1:100,000.

10.57 Injected 1 cc. of the dilute adrenalin solution into the base of the right ear of rabbit A.

10.58 Injected 1 cc. of the dilute adrenalin solution into the base of the left ear of rabbit B. Within one minute the injected ear was paler in each rabbit, but bloodvessels, central artery included, were by no means obliterated. Injected ear vessels were moderately dilated, but never reached the powerful engorgement seen in the normal ear. Excitation caused blanching of both ears.

11.05 Same; injected ears paler than normal ears, the difference is well marked. But the central artery of the injected ear is moderately dilated (about one-half to one fourth of size of the central artery of the normal ear).

11.22 Smaller vessels (arterioles) not as numerous in injected ear as in normal ears. Well marked difference between pale injected ears and flushed normal ears.

12.04 Well marked difference still present; vessels of injected ear never dilated to the same extent as in normal ears and smaller vessels never as numerous.

1.30 p.m. Rabbit A. Both ears blanch on excitation; then normal ear usually flushes first; later injected (right) ear begins to dilate until

central artery is practically as large as that of normal ear, but injected ear nevertheless is paler, on account of the smaller arterioles not dilating as well as those of the normal ear.

Rabbit B. Difference between the two ears stronger than in rabbit A; injected ear does not fill up to the same degree as the normal ear, and central artery is always smaller.

3.05 Injected ears flush now about as strongly as control ears, often begin to flush before the normal ear after excitation. Central artery of injected ears as wide as those of control ears. In rabbit B upper fifth of central artery (including bifurcation) does not dilate well. This rabbit now responds but slightly to excitation: practically no blanching occurs.

5.40 Rabbit A. After excitation both ears pale at first then right (injected) ear flushed maximally before left, normal ear; after a considerable period normal ear dilated to same extent as injected ear. Well seen in central artery.

Rabbit B. Both pale at first examination, then both ears begin to gorge at approximately the same time; injected ear a bit more perhaps. Upper fifth of central artery now well dilated (was constricted before), while lower part of central artery contracts well.

Summary. Experiment 10 consists of a double observation made simultaneously on two rabbits under exactly similar conditions. The constricting effect appeared swiftly in both animals, one minute after the injection. The constricting effect however was at no time maximal, and the paleness depended largely upon the disappearance of the arterioles and less upon the constriction of the central artery which was at no time excessive. The constricting effect lasted several hours. But in one animal the vessels of the injected ear showed an unmistakable tendency to dilatation four hours after the injection, while at that time in the other the condition of the vessels was on the borderline. In animal B. the constricting effect seemed to be a trifle more persistent than in animal A.

Many more experiments of this sort were made. The results were essentially the same and are sufficiently illustrated by the experiments cited in the foregoing pages.

A number of experiments were made in which either the sympathetic nerve alone was cut or the superior cervical ganglion alone was excised or in addition the large ear nerve was sectioned several days before the adrenalin experiment was made. The blood-

vessels of the ears of rabbits so prepared were of course wider than in normal animals. But the course of the effect of an injection of adrenalin in these animals with denervated ear vessels was not materially different from that observed and described in the experiments on ear vessels with intact innervation.

We shall mention that in the various series of experiments we met with one only in which an injection of 0.3 cc. adrenalin at the inner margin of the base of the ear caused no perceptible effect upon the bloodvessels of that ear. The connective tissue in that place is dense and does not permit the spreading of the fluid to adjacent places. In all the other experiments the injection of adrenalin had a definite effect, the nature of which was invariably constriction of the bloodvessels.

DISCUSSION

Before discussing our results in detail, we wish to make a brief remark upon the restricted value of the bloodpressure curve as a means of studying the action of epinephrin. The rise of bloodpressure following the intravenous injection of epinephrin gives information as to the constriction of the bloodvessels only by inference and not by direct observation, and does not tell the whole story. A rise in bloodpressure indicates that the vascular bed became smaller, which can be accomplished only by some constriction of bloodvessels. But the degree of constriction may vary in various parts of the body and so may its duration. Furthermore, an agent which causes a vasoconstriction in one part of the body may cause a vasodilatation in another part. The rise of bloodpressure is only an expression of the fact that the algebraic sum of the several effects of a given agent amounts to a diminution of the vascular bed of a certain degree and duration. The rise of bloodpressure, measured, as it is done, in the large arteries of the body, brought about by an intravenous injection of epinephrin, shows that the resultant is a vasoconstricting action. It does not indicate that there is another effect, and does not indicate what is the actual state of the bloodvessels of the heart, the kidneys, the bladder, the lungs, the brain, or of other parts of the body.

The intravenous injection of epinephrin which brings out the striking rise of bloodpressure, brings out at the same time another striking and puzzling fact, namely, the brevity of the rise. The duration of the rise varies with the injected dose and with the species of animals into which the injection is made (7). In rabbits in which the rise is the longest, the pressure returns to normal in seven minutes at the latest. As a rule, after return to normal the blood contains no demonstrable epinephrin.

At various times we met with experimental facts which showed that in some modes of administration the duration of the effect upon the bloodvessels is longer than the one observed after intravenous injections of epinephrin. In our second study of absorption from the intramuscular tissues (8) a tracing is reproduced which shows that about half an hour after the injection of adrenalin the blood pressure had not yet returned to normal. In our studies on the intraspinal injection of epinephrin (9) a tracing is published in which seventy-five minutes after an intraspinal injection the bloodpressure had not yet come down to the original level. In both instances the epinephrin was injected into places other than the vascular bed.

In the series of experiments reported in this paper the condition of the vessels was studied in a single organ, the ear; the injections were made into that organ itself, subcutaneously, and the results were reached by a direct observation of the bloodvessels and the tissues under normal conditions. In these experiments we have seen nothing but constriction of the bloodvessels and even such a small dose of adrenalin as 0.01 mgm. caused an unmistakable constriction.

The outstanding fact in our results is the remarkable duration of the constricting effect. There was practically no instance in which the evident constriction of the vessels subsided in less than three hours and sometimes it persisted as long as eight hours. It must be emphasized that the maximum duration of bloodpressure rise by intravenous injection lasts seven minutes; the duration of constriction of bloodvessels by local subcutaneous injection of epinephrin lasts 180 to 480 minutes.

In general, it may be stated that the duration of the constricting effect of adrenalin increased with the size of the injected

dose. When injected in the same place and under the same conditions the constricting effect lasted definitely longer after 1 cc. for instance, than after 0.5 cc. A very important factor is the place of injection. Compare for example the very long duration of constriction in experiment 8 (eight hours) in which 0.4 cc. was given near the central artery, on each side of it, with the duration in experiment 7 in which 1 cc. was injected midway between the central artery and the margin of the ear.

What was said for the duration holds true also, although perhaps to a less degree, for the intensity of the effect. The ear was paler and the vessels were more constricted, when the injected dose was larger and when the injection was made near the central artery.

The site of the injection had a special influence upon the latent period and the development of the constricting effect. When the injected fluid spread to the central artery, or, still more, when half of the quantity of solution was injected near each side of the central artery, paleness of the ear set in at once and in most instances with maximum intensity. In experiment 4 in which 0.5 cc. was injected midway between the central artery and the margin of the ear, more than ten minutes passed before there was any sign of constriction, and during the entire experiment the effect was moderate. But in experiment 5 in which 0.25 cc. was injected on each side of the central artery and near it, the entire ear, from the base to the tip, became pale in less than one minute. The pallor was intense and the effect lasted about five hours. On the other hand in experiment 6 in which 0.3 cc. of adrenalin was injected on each side of the central artery but away from it and near the margin of the ear, the first constricting effect was noticed as late as twenty minutes after the injection, although gradually the effect became well pronounced and was of considerable duration. Or compare the following results. In experiment 7, 1 cc. of adrenalin was injected midway between the central artery and the margin of the ear. Eleven minutes passed before there was any sign of constriction; it then developed gradually but reached a considerable intensity and persisted with a gradually diminishing degree

for about five hours. In experiment 8, 0.4 cc. was given on each side of the central artery. The pallor developed practically immediately, the constriction of the central artery was seen traveling up to the tip within one minute and the effect was very strong and persisted for about eight hours.

Oliver and Schafer (10) assumed that medium sized arteries are not constricted by an adrenal extract and believed that they participate by passive dilatation in the oncometric rise of the curve obtained from an extremity. Langley (11), on the other hand, thought that adrenal extract causes a constriction of the medium sized vessels. In our experiments there was not the slightest doubt as to the constriction of the central artery which must be considered as middle sized. When a larger dose is injected and the injection is made at the base near the vessel, the entire artery becomes constricted at once and later may be invisible. (Sodium chloride, when injected at the base, exerts no effect). With smaller doses and when injected at some distance from the artery, the constriction is gradual. In this case a chain of short fine sections of the artery, containing little columns of blood, may be seen repeatedly moving upward. The same is often seen when the constriction is on the point of receding. The relaxation of the central artery always sets in ahead of a noticeable subsidence of the pallor of the ear.

As far as we know, no study exists as to the action of epinephrin upon veins *in situ*. Gunn and Chavasse (12) stated recently that rings of veins contract when immersed in a solution of epinephrin. In our experiments we could have no doubt that the veins often become constricted; but the constriction of veins set in later than that of the central artery or of the arterioles. This was definitely seen even when the constriction of the central artery set in rapidly. When the development of the arterial constriction was a slow process, at first the blood in the veins became dark and the pale ear had a bluish hue; later the veins became empty; then they narrowed, began to lose their outlines and finally became invisible. In intense constrictions the effect extended also to the marginal veins.

The arterioles are seen as "ribs" coming from the central artery and a net work of fine vessels is seen between the rib in the more or less transparent areas on the sides of the central artery. When the injection was made near the artery, the arterioles disappeared practically simultaneously with the constriction of the central artery. When the injected fluid reached the artery only on one side the suddenness of the onset and the intensity of the effect upon the arterioles was greater on the side in which the injection was made. Apparently the central artery is bound to the surfaces by some dense tissue which offers resistance to the spread of the fluid. As the constricting effect progressed, not all the small arterioles disappeared at the same time, the "ribs" seemed to persist longer than the vascular net work between them, and the ribs, too, disappeared only one after another. The decrease in the number of arterioles became evident before the constriction of the central artery made its definite appearance. (The first manifestation of an impairment of the central artery was its failure to dilate promptly after the constriction which follows an excitation or a struggle.)

The spaces in the ear between the arterioles are usually pink which is due to the blood within the capillaries. When the constricting effect developed slowly the interarterial spaces seemed to lose their pink color before the arterioles began to disappear.

The effect of the injection of adrenalin became often manifest by some other signs than change or loss of color. *The ear became definitely cooler than the other ear, was heavier and hung down while the other ear was erect. The ears were moved very little.* With regard to the temperature, it was sometimes noticed that the injected ear felt cooler than the normal ear before it was recognized that it was paler.

We shall recapitulate some points. An injection of 0.3 cc. of adrenalin into the tip of the ear caused a rapid onset of a fairly intense paleness of the ear which was of long duration. An injection of 0.3 cc. of normal salt solution also caused a rapid onset of paleness which however lasted only a short time. The early appearance of pallor in either case we ascribed, at least in part, to a mechanical factor resulting from the density of the

connective tissue in the injected spaces. In the single failure mentioned above, the injection was made close to the margin of the ear where the connective tissue is quite tense which prevents the fluid from spreading readily to adjacent spaces. The rapid effect from an injection in the tip was probably due mainly to the close proximity of the bifurcation of the central artery. The fluid ran down close to the artery which was probably only *compressed* by the saline but became *constricted* by the adrenalin. It should be recalled that the injection of the saline caused the development of a ridge along the central artery, while no ridge appeared when adrenalin was injected.

When saline was injected at the base of the ear even when the quantity was 1 cc., there was no effect upon the calibre of the vessels. When adrenalin was injected at the base there was an unmistakable constricting effect. When the injection was made on both sides of the central artery and near it, the paleness of the entire ear and the constriction of practically all the vessels developed rapidly and was fairly intense and of long duration. No ridge was ever observed to develop along the artery. When the injection was made midway between the artery and the margin of the ear, the effect developed more slowly and the slower the further away from the margin the injection was made. In this case some paleness developed in a good portion of the ear before the central artery showed any constriction; the latter progressed slowly along the central artery.

Epinephrin is readily destroyed in an alkaline solution. With this in mind and with the fact of the alkalinity of the tissues, several authors ascribed the rapid decrease of the bloodpressure after an intravenous injection of epinephrin to the escape of the latter into the body tissues and its destruction there. *Our experiments dispose completely of this explanation.* On the contrary, adrenalin injected directly into the tissues remained strongly active for many hours. It seems that our experiments rather favor the assumption that the place of destruction of epinephrin is within the bloodvessels. The constriction of the arterioles and capillaries prevents the rapid entrance of the epinephrin into the lumen of the vessels and thus permits its

long continued efficient action. This presupposes that the constriction of the vessels in our experiments is caused by the epinephrin *reaching the tunica muscularis directly through the adventitia and not through the intima after entering the lumen of the vessels through the capillaries*. This deserves to be especially emphasized since some writers assume that all actions of epinephrin upon the bloodvessels are effected from the lumen of the vessels. Patta (13), for instance, claimed that the blood-pressure effect of subcutaneous or intramuscular injections is attained simply by the needle inadvertently entering a bloodvessel. In our previous experiments we (14) have taken special precaution to eliminate such accidents. In the present experiments the unusual length of the duration of the constricting effect eliminates the consideration of an intermediary action through the lumen of the vessels.

As to the rapid disappearance of the epinephrin within the blood, the experiments of Tatum (15) are interesting. He found that epinephrin disappears rapidly from a solution when arterial wall is present. But the solution has to be oxygenated. However, we shall not enter here into a discussion of the cause of the rapid disappearance of the epinephrin within the blood as well as the cause of the final disappearance of the constricting effect in our experiments.

The long duration of the constriction in our experiments is an interesting illustration of the low fatigability of the tunica muscularis. It is also a noteworthy fact that the paleness which persisted for many hours and which sometimes was extreme, never led to necrosis or even to serious temporary injuries of the ear. While the place of injection was found sometimes next day to be slightly inflamed and edematous, it soon became normal again. Practically in all of our experiments the bloodvessels of the injected ear manifested next day a tendency to dilatation which was definitely greater than in the vessels of the normal ear. Another point of interest is that in many experiments about simultaneously with the onset of the constricting effects observed in the injected ear, a greater dilatation of the vessels of the other ear

made its appearance. This dilatation was not of very long duration. Both last mentioned phenomena may be significant; but we shall not attempt to analyze here their possible meaning.

SUMMARY

Rise of bloodpressure is only a circumstantial evidence for the constriction of bloodvessels in some part of the vascular bed which is sufficient to overbalance any other effect. An ocular study of the bloodvessels in the rabbit's ears permits a direct observation of the behavior of all the larger vessels in that organ. The experiments reported in this paper have shown conclusively that a subcutaneous injection of epinephrin in the ear of rabbits causes a constriction of all the vessels of that ear. The constriction is quite intense; but the outstanding feature is its very considerable duration—between three to eight hours. The rise of bloodpressure from an intravascular injection of epinephrin is at the utmost seven minutes.

The latent period which passes between the time of the injection and the onset of the constriction is the longer the further away the injection is made from the central artery. Injections made near to the central artery and on both sides of it cause practically an immediate paling of the entire ear and constriction of the central artery with all its branches and of the veins.

In subcutaneous injections of the ear the epinephrin apparently reaches the muscular sheath through the adventitia and not through the intima from the lumen of the bloodvessels.

An ear which received a subcutaneous injection of epinephrin is cold, heavy, and is infrequently moved by the animal.

When the constriction passes off the bloodvessels which were subjected to this effect show later a tendency to the opposite effect, to vasodilatation.

A subcutaneous injection of adrenalin in one ear which causes a constriction of the vessels of that ear seems often to cause at about the same time a dilatation of the vessels in the other ear; the dilatation is not of long duration.

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STUDIES OF CHRONIC INTOXICATIONS ON ALBINO RATS IV.

FLUORID, CHLORID AND CALCIUM (INCLUDING SODIUM FLUORID, SODIUM CHLORID, "PHOSPHATE ROCK," CALCIUM PHOSPHATE (PRECIPITATED) AND CALCIUM CARBONATE (PRECIPITATED))

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INTRODUCTION

Fluorids have considerable scientific interest. They have been credited with producing interesting chronic intoxications, which might possibly be connected with the formation of insoluble calcium salts.

The subject also has a practical bearing, from the use of sodium fluorid as preservative; and from the accidental presence of fluorin compounds in "phosphate" baking powders, prepared from "phosphate rock."

The experiments were conducted by the methods described in the first paper; adding to the food in the first place a very pure specimen of sodium fluorid, and then comparing the effects with those of phosphate rock, sodium chlorid, calcium phosphate and calcium carbonate, in order to study the rôle of various possible factors in the fluorid intoxication. In a number of experiments, the drug period was followed by a drugless period, to learn whether the damage would be irremediable.

The present paper reports the results on growth and food consumption. The tissues are being studied microscopically by Dr. Maurice Richardson, and material has been reserved for quantitative estimation of fluorin retention.

A. SODIUM FLUORID

Dosage of sodium fluorid. This is shown in table 1. The weekly variations parallel the food consumption, and can be gathered from the charts of food consumption, if desired. The experiments range between 0.15 and 151 mgm. of NaF per kilogram of body weight, daily for one to four months. In man, this would correspond to about 9 mgm. to 9 gram or $\frac{1}{6}$ to 150 grains per day.

TABLE 1
Mean dosage of fluorid, and duration of experiments

CONCENTRATION OF SODIUM FLUORID	EXPERIMENT NUMBER	NUMBER OF ANIMALS IN EXPERIMENT	DURATION	MEAN DOSAGE OF FLUORID PER KILOGRAM OF RAT PER DAY
<i>per cent</i>			<i>weeks</i>	<i>mgm.</i>
0.0002	15	10	9	0.15 (0.15-0.16)
0.002	14	10	9	1.50 (1.4-1.6)
0.01	28	5	5	7.90 (7.5-8)
0.02	67	2	21	16.00 (11-39)
0.02	13	10	14	14.80 (12.4-17.2)
0.04	5293	3	18	19.80 (7.6-54.4)
0.23	12	7	10	151.00 (89-255)
$\frac{1}{2}$ NaF, $\frac{1}{2}$ normal				
0.05	66	4	24	42.00 (37-44)
0.1	3950	6	15	70.50 (65-76)
0.24	74	6	19	86.40 (72-96)

In the last three experiments of the table, the rats had equal access to poisoned and unpoisoned food. The poison in these cases was therefore consumed quite voluntarily.

The dosage of sodium fluorid was selected from two points of view: (1) the quantity used in investigations of chronic fluorid intoxications by other workers; and (2) the quantity likely to be consumed by man in the form of baking powder.

Quantities of sodium fluorid used by other investigators. These are arranged in ascending order.

NaF, 0.00062 per cent of food. Osborne and Mendel, 1913, added this to their food "IV." Rats thrive excellently on this food.

HF, equivalent to 0.35 to 2.5 mgm. of NaF per kilogram of body weight, equivalent to rat experiments, with 0.0002 to 0.002 per cent NaF in food. Woakes, 1881, treated 20 goiter patients with hydrofluoric acid, 0.5 to 2 drams of 0.5 to 1 per cent (about 10 to 80 mgm.) per day, for six months to two years. No ill effects were observed.

NaF, mean of 1 to 1.5 mgm. per kilogram per day, equivalent to rat experiments with 0.002 per cent NaF in food. Schwyzer, 1914; Intramuscularly, 3 rabbits, daily for nine to thirteen weeks. Animals appear to have remained in good condition, with mean weight only 10 per cent less than controls; but claims somewhat indefinite signs of disturbed function of bone-marrow (mononuclear leucocytosis).

NaF, about 4 mgm. per kilogram, equivalent to rat experiments with 0.005 per cent NaF in food: Tappeiner quotes Rabuteau as taking 0.25 gram by mouth, without any remarkable result except salivation lasting one and one-half hours (nausea from salt-action?).

NaF, about 5 to 15 mgm. per kilogram per day, equivalent to rat experiments with 0.02 per cent NaF in food. Schulz, 1889, injected an adult rabbit with 10 to 40 mgm. hypodermically, daily for twenty-five days. No effects noted.

NaF, about 10 to 30 mgm. per kilogram per day, equivalent to rat experiments with 0.02 per cent NaF in food. Schwyzer, 1903, fed rabbits with 30 mgm. per day, for ten to twenty days. No symptoms during first days. Then gradually considerable anorexia and emaciation. Killed at the end of the period.

NaF, about 20 to 40 mgm. per kilogram per day, equivalent to rat experiments with 0.04 per cent NaF in food. Schulz, 1889, administered in food to a dog 0.2 to 0.4 gram per day. Emesis during first few days; emaciation. Died in two weeks.

NaF, about 8 to 80 mgm. per kilogram per day, equivalent to rat experiments with 0.01 to 0.2 per cent NaF in food. Brandl and Tappeiner, 1891, fed dog with above dosage for twenty months. Above 50 mgm. equivalent to 0.04 to 0.1 per cent NaF in food; diarrhea and nausea occurred at times. Large quantity of fluorid were deposited in bones.

NaF, about 50 to 200 mgm. per kilogram, equivalent to rat experiments with 0.23 per cent NaF in food. Schulz, dogs, oral: prompt emesis, with recovery.

NaF, about 100 to 160 mgm. per kilogram per day, equivalent to rat experiments with 0.23 per cent NaF in food: Rabbits, hypodermic for three days. One died on fifth day; the other developed actinomycosis.

NaF, about 100 to 200 mgm. per kilogram equivalent to rat experiments with 0.23 per cent NaF in food. Rabbits, hypodermic, acutely fatal dose. Symptoms as observed by Tappeiner.

NaF, about 150 mgm. per kilogram, equivalent to rat experiments with 0.23 per cent NaF in food. Tappeiner, 1889, determined this as the average hypodermic or intravenous fatal dose for mammals. There is considerable salivation and lachrymation; increase of respiration; often emesis; somnolence; epileptoid convulsions alternating with coma; and death in an hour, by arrest of respiration. Early rigor mortis.

NaF, about 500 mgm. per kilogram, equivalent to rat experiments, with three times 0.23 per cent NaF in food. Tappeiner determined this as the average acutely fatal oral dose for mammals.

Quantity of fluorid likely to be consumed in baking powders. According to information obtained from the United States Bureau of Chemistry, "phosphate rock" is used to some extent in the production of the phosphates used in the manufacture of baking powders. This is likely to contain fluorids, probably in a form similar to apatite. "Ordinarily, such rock contains from 0.5 per cent to 15 per cent of fluorine. The finished baking powders are made from the acid calcium phosphate containing in the neighborhood of 0.04 per cent of fluorin; but, if carelessly manufactured, i.e., if calcium acid phosphate is used, they may contain as much as 0.5 of 1 per cent."

Dr. E. W. Schwartze (personal communication) calculates (by analogy with the Referee Board data, in Department Bulletin 103, "Alum in Foods") that the daily intake of fluorin through the use of baking powders would approximate 0.35 to 2.84 mgm. if the powders contain 0.04 per cent of fluorin; or 4.45 to 35.55 mgm. if the powders contain 0.5 per cent of fluorin. In terms of NaF, this would represent an extreme range of 0.77 to 78.1 mgm. ($\frac{1}{85}$ to $1\frac{1}{5}$ grain) per man, 0.13 to 1.3 mgm. per kilogram of body weight. This is in the lowest range of the dosage of our rat experiments (0.0002 per cent).

Effects of sodium fluorid on growth. These are shown in table 2 and figures 1, 2 and 3. In the table, the data are given for the end of the experiments, and for other periods when important changes occurred. It should be recalled that the variations of

the growth of unpoisoned animals from the standard growth range between -1 and $+1.8$ per cent per week, with a median of $+0.13$ per cent. The corresponding number for the fluorid rats are in the last column.

Well within this normal range is the growth of the rats that were fed on 0.0002 to 0.01 per cent of NaF, i.e., up to 7.9 mgm. of NaF of rat per day, for five to nine weeks.

TABLE 2
Effects of sodium fluorid on growth

CONCENTRATION OF SODIUM FLUORID IN FOOD	DOSAGE OF NaF PER KILOGRAM OF RAT PER DAY	EXPERIMENT NUMBER	DURATION	OBSERVED WEIGHT	NORMAL WEIGHT	DIFFERENCE	DIFFERENCE OF NORMAL WEIGHT	DIFFERENCE PER WEEK
<i>per cent</i>	<i>mgm.</i>		<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
0.002	0.015	15	9	185	185	0	0	0
0.002	1.5	14	9	168	172	-4	-2.3	-0.25
0.01	7.9	28	5	124	124	0	0	0
0.02	14.8	13	{ 10	188	198	-10	-5.0	-0.5
			{ 14	194	225	-31	-13.6	-0.97
0.02	16.0	67	21	148	185	-37	-20.0	-0.95
0.04	19.8	5293	{ 15	283	265	+18	+6.7	+0.44
			{ 18	225	260	-35	-13.0	-0.7
0.05*	42.0	66	12	180	232	152	-22.0	-1.8
0.1*	70.5	3950	15	180	234	-54	-23.0	-1.5
0.24*	86.4	74	19	170	248	-78	-31.0	-1.6
0.23	151.0	12	{ 5	62	112	-50	-44.6	-8.9
			{ 10	58	150	-92	-61.0	-6.1

* In these experiments, the rats had equal access to normal food.

With concentrations of 0.02 to 0.04 per cent, or 15 to 20 mgm. per kilogram moderate but definite interference of growth develops after a time. In one of the groups (no. 67) the retardation begins early and progresses steadily. In the other two groups (nos. 13 and 5293) the growth remained normal for ten to fifteen weeks, and then suddenly declined.

With concentrations of 0.05 to 0.23 per cent or 42 to 151 mgm. per kilogram, there is very marked retardation of growth, starting with the administration, and increasing with the dose from 1.5 to 9 per cent of body weight per week. The total

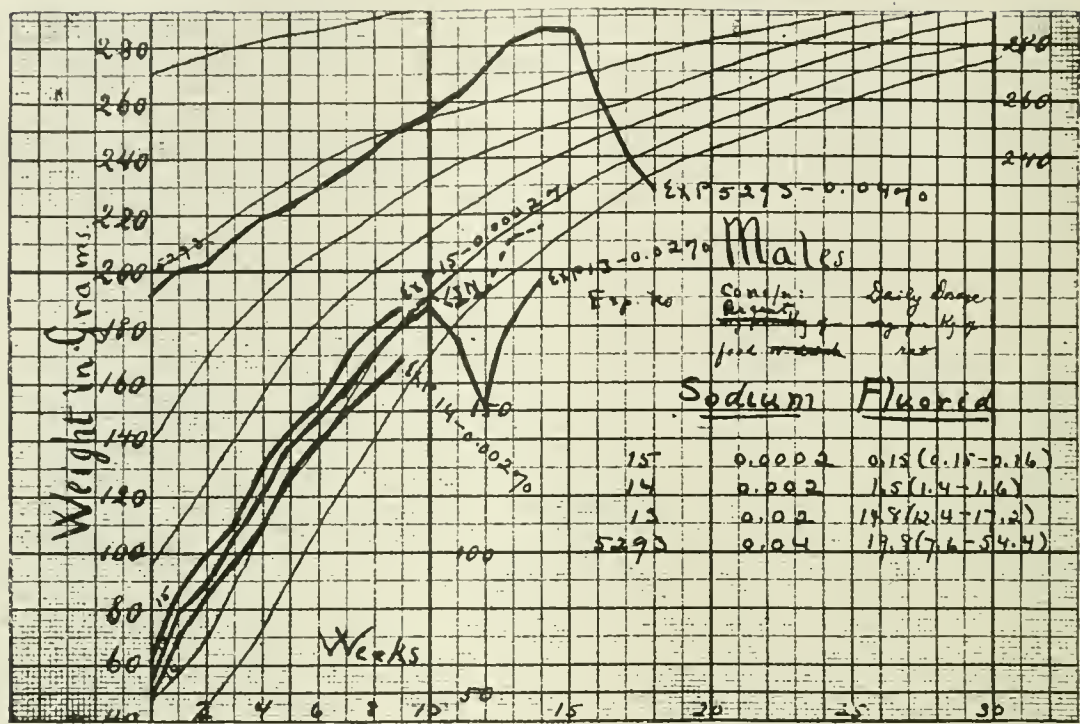


FIG. 1. SODIUM FLUORIDE ON GROWTH OF MALE RATS

The light lines represent standard curves of normal growth rate. The heavy lines represent the growth rate, while the rats were being fed on poisoned food. The dotted lines correspond to the periods when the rats (usually half of the group) were replaced on unpoisoned food.

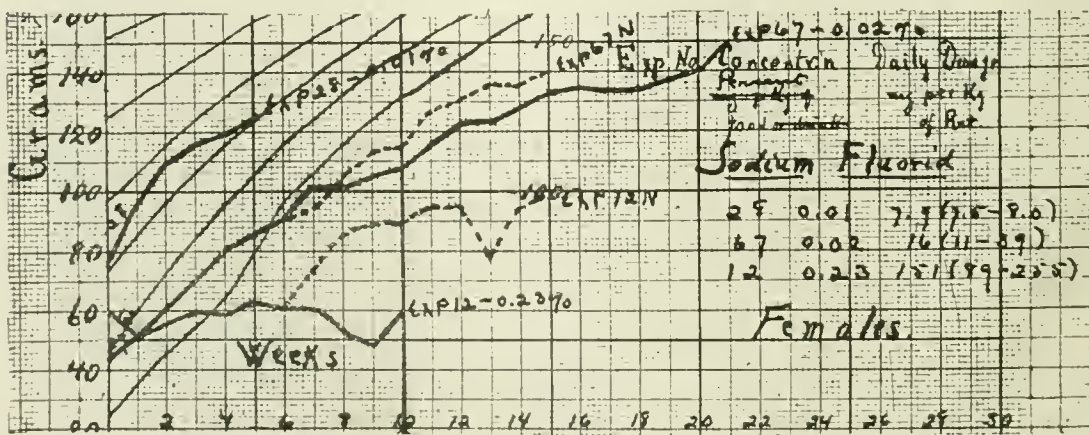


FIG. 2. SODIUM FLUORIDE ON GROWTH OF FEMALE RATS

retardation in the five to twenty weeks of the experiments ranges from 22 to 61 per cent of the normal weight. The animals had free access to unpoisoned food in three of the experiments.

After-effects on growth. The dotted lines on the growth curves, figures 1 to 3, present rats which were placed on unpoisoned food, after their growth had been checked by the fluorid food, as represented by the solid lines.

In every case, including experiment 12, with the largest dosage, the growth rate is much accelerated over that during the

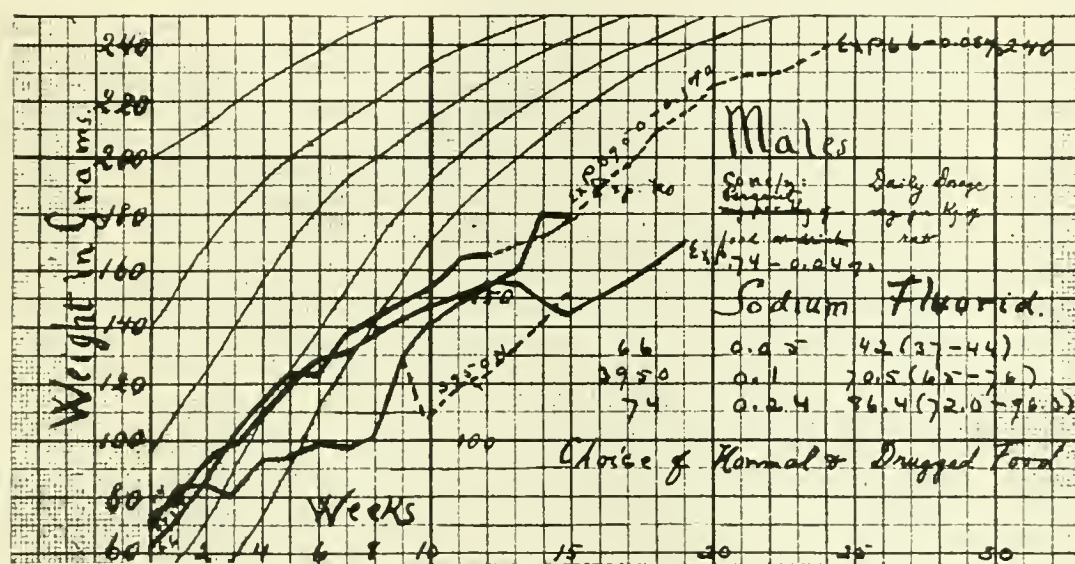


FIG. 3. SODIUM FLUORIDE ON GROWTH OF MALE RATS

In the experiments of this chart, the animals had equal access to poisoned and unpoisoned food.

poison period; but it generally fails to reach the normal rate, and still less does it make up for the checked growth during the drug-period. In other words, the damage done by the fluorid appears to be irreparable, and the animals that were stunted by the fluorid remain stunted for indefinite periods.

Experiment 66 in figure 3 is a good example. The animals ate food averaging 42 mgm. of NaF per day for twelve weeks, when their weight was 180 grams, i.e., 52 grams or 22 per cent below standard (232 grams). They were then fed for another

twelve weeks on unpoisoned food. Their weight was now 238 grams, i.e., 30 grams or 11 per cent below standard. With the larger dosage, the failure to make good the initial loss is more striking, especially in experiment 12 of figure 2.

Effects of fluorid on food consumption. These are shown in table 3 and in figures 4, 5, and 6. It should be remembered that the variations of unpoisoned rats from the standard range between -0.08 and $+19$ per cent of the standard food figures.

With the small dosage of fluorid (0.15 to 8 mgm. per kilogram of rat per day; experiments 15, 14 and 28) the food consumption is practically unaffected. It ranges from -2.2 to $+11$ per cent of the standard. The $+11$ per cent applies to the largest dosage of the group.

With the median dosage (15 to 20 mgm. per day), two series (nos. 13 and 67) show moderate but distinct diminution of food consumption (by 6 and 8 per cent), corresponding to their growth deficit (1 per cent). In the third series (5293) the food consumption is within the normal range ($+7.8$ per cent) and in this the growth-deficit (0.7 per cent) was also within the normal range.

With the largest dosage (86 to 151 mgm. per day), the two series (nos. 74 and 12) show markedly diminished food consumption (13 and 44 per cent), corresponding to the check of growth (1.6 and 6.1 per cent).

With the intermediate dosage of 42 and 71 mgm. in the two series (66 and 3950), the food consumption is not parallel to the growth changes. Both show marked check of growth (1.8 and 1.5 per cent); but the food consumption is normal in one, and much above normal in the other (no. 3950). The latter is apparently not a technical error, for there is a certain correspondence between the details of the growth and food curves.

Summarizing the data, there is, with a few striking and unexplained exceptions, a general parallelism between the food intake and the growth. This parallelism is generally quite close, and reflects even daily variations; as in experiment 5293, when the growth curve and food curve both decline sharply on the fifteenth week (figs. 1 and 5); and similarly in the tenth week of experiment 13 (figs. 1 and 4).

Similarly, when the animals are transferred to unpoisoned food, increase of appetite and of growth again are very closely parallel.

It may be concluded that sodium fluorid in doses above circa 10 mgm. per kilogram per day diminishes both food consump-

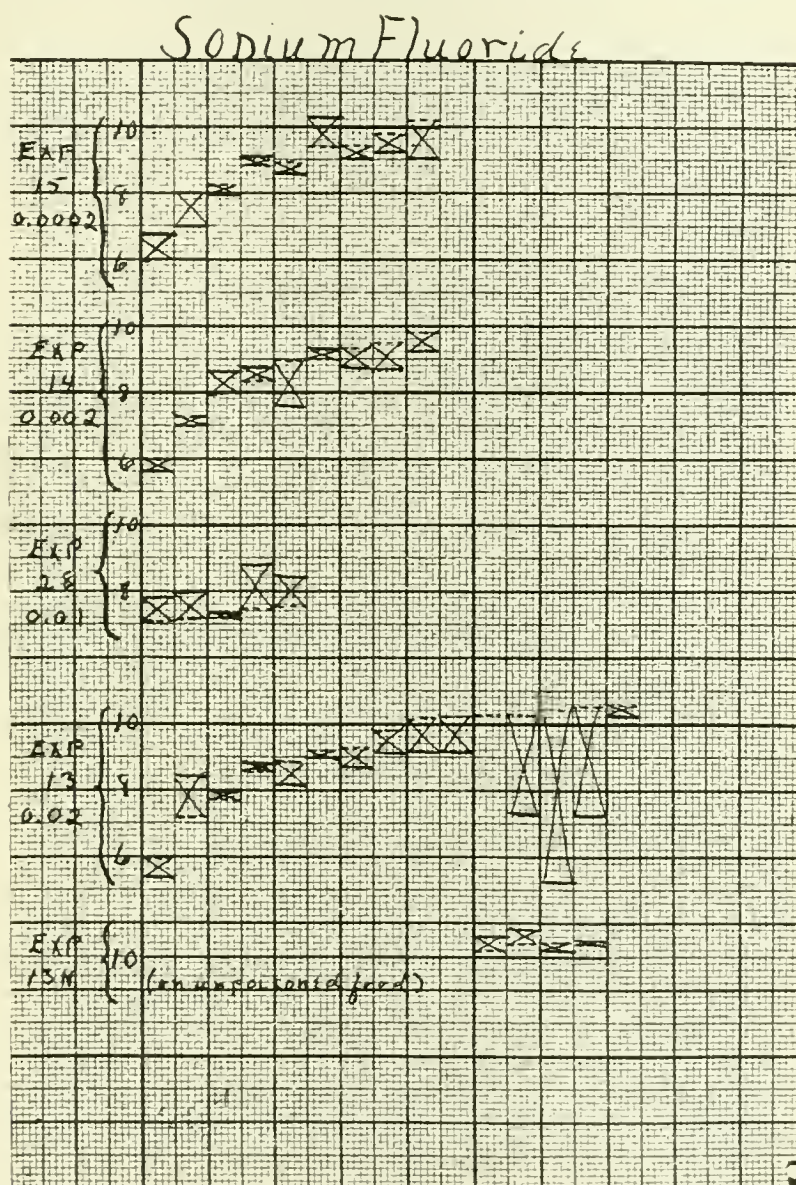


FIG. 4. SODIUM FLUORID ON FOOD CONSUMPTION

Concentrations of 0.0002 to 0.02 per cent of NaF. The numbers above the experiments are the dates of the observations. The numbers to the left represent the grams of food consumed daily per rat. The dotted horizontal lines represent the standard food consumption of rats of that size. The solid horizontal lines, joined to the dotted lines by crossed lines, represent the actual food consumption.

Sodium Fluorid.

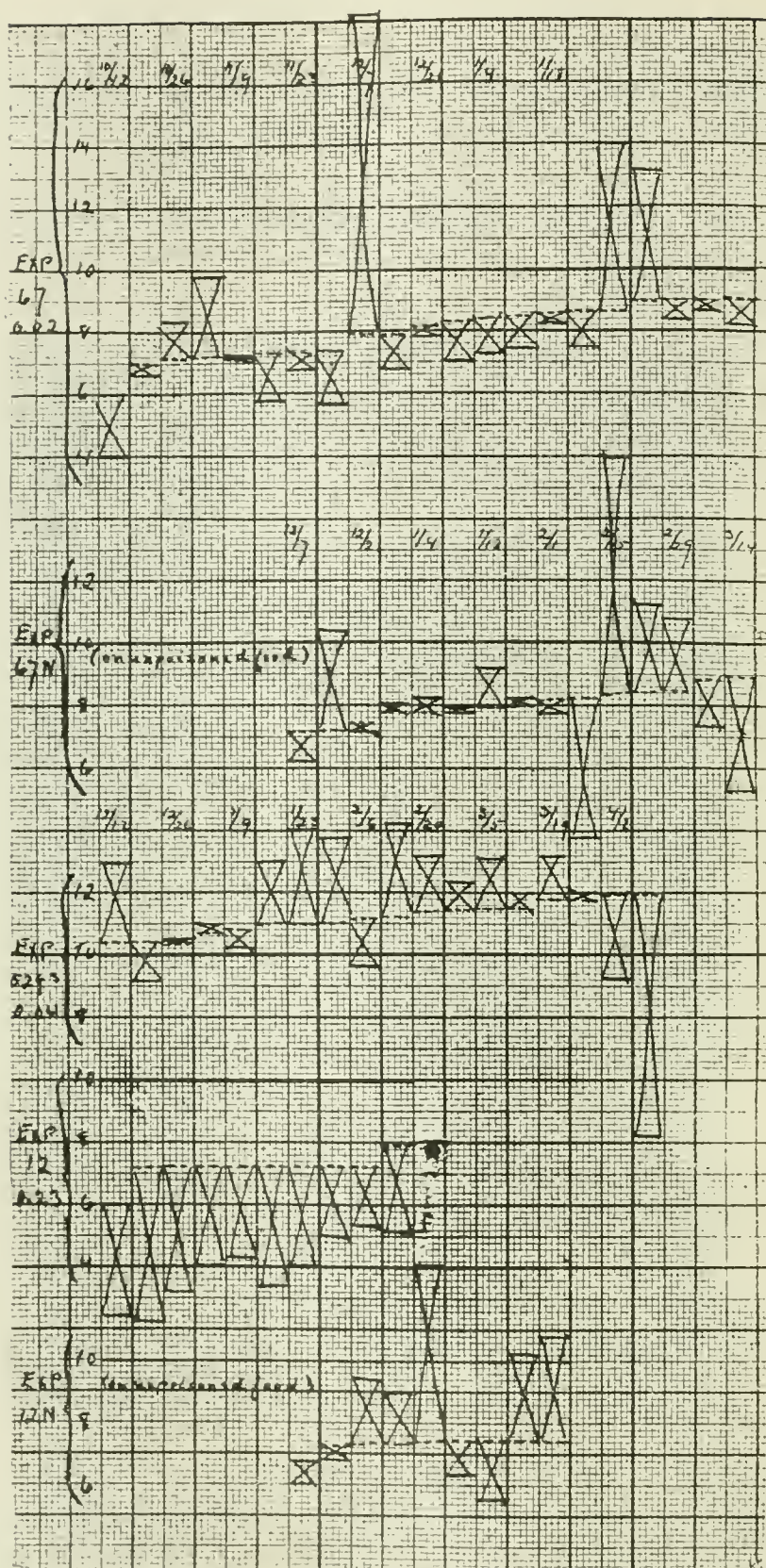


FIG. 5. SODIUM FLUORID ON FOOD CONSUMPTION
Concentrations of 0.02 to 0.23 per cent

tion and growth; and that the two conditions are generally parallel. Since, however, one may occur without the other, they do not bear an invariable causal relation. Both are probably distinct consequences of an underlying disturbance; although each will, of course, tend to exaggerate the other.

Our results permit us to answer definitely the question, whether the diminished food consumption is due to a distaste for the fluorid poisoned food? For this purpose, three series of animals were offered equal access to poisoned and unpoisoned food.

TABLE 3
Food consumption

NaF IN FOOD	EXPERIMENT NUMBER	DURATION OF EXPERIMENT	GROWTH, MEAN DIFFERENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMPTION MEAN DIFFERENCE FROM NORMAL STANDARD	
				Grams per rat per day	Per cent
<i>per cent</i>		<i>weeks</i>			
0.0002	15	9	0	-0.2	-2.2
0.002	14	9	-2.5	-0.3	-3.3
0.01	28	5	0	+0.8	+11.0
0.02	13	14	-0.97	-0.6	-6.0
0.02	67	21	-0.95	-0.5	-6.0
0.04	5293	18	-0.7	+0.8	+7.8
0.05	66	12	-1.8	0	0
0.1	3950	15	-1.5	+4.25	+44.0
0.24	74	19	-1.6	-1.4	-13.0
0.23	12	10	-6.1	-3.25	-44.0

* That is, figure of preceding column $\times 100 \div$ standard mean food in grams.

Figure 6 shows that with concentrations up to 0.1 per cent, the rats ate the poisoned food as readily as the unpoisoned. Similarly, when replaced on unpoisoned food the appetite did not show a marked increase, as would be expected if the poisoned food had been distasteful to them (fig. 5, experiment 67, 67N; fig. 6, experiment 3950).

However, with the maximal concentration of 0.23 per cent of NaF, there was distinct discrimination against the fluorid food (fig. 6, experiment 74; fig. 5, experiments 12 and 12N).

We conclude, therefore that diminished food consumption is not due to distaste for the flavor of the fluorid food, if the concentration does not exceed 0.1 per cent of NaF; but that with 0.23 per cent of NaF, the taste plays a part.

Mortality under sodium fluorid. This is shown in table 4. Even with the large doses of 71 to 86 mgm. of NaF per kilogram per day, the total fatalities are only 4 animals out of 12, i.e., 33 per cent. This is practically the same as in the control series

TABLE 4
Mortality

MEAN DOSAGE OF NaF PER KILOGRAM OF RAT	EXPERI- MENT NUMBER	NUMBER OF ANIMALS	WEEKS OF FATALITIES	TOTAL DURATION OF FEEDING WITH NaF	FATALITIES
<i>mgm.</i>					<i>per cent</i>
0.15	15	10	0	9	0
1.5	14	10	0	9	0
8.0	28	5	0	5	0
15.0	13	10	0	14	0
16.0	67	2	17	21	50
20.0	5293	3	0	18	0
42.0	66	4	0	12*	0
71.0	3950	6	3, 5, 5	15	50
86.0	74	6	8	19	16
151.0	12	7	1, 2, 2, 10, 10, 10, 11	11	100

* These animals were observed for a total of twenty-four weeks, without any fatalities.

with NaCl, described in section B. It is therefore very doubtful whether the fatalities should be referred to the fluorid. This is rendered further improbable by the fact that the deaths all occurred early in the experiments, and the fatality did not increase with the duration of the feeding, as would be expected if it were due to the drug.

Undoubted fatality, namely 100 per cent, occurs with the highest dosage, i.e., 151 mgm. per day. The animals died either in the first and second weeks, or in the tenth and eleventh week.

B. SODIUM CHLORID

Introduction. Section A of this paper showed that sodium fluorid produces harmful effects on growth and food consumption when added to the food in concentrations of 0.02 to 0.04 per cent, or 15 to 20 mgm. per kilogram of rat per day; and markedly harmful effects with 0.05 to 0.23 per cent, or 42 to 151 mgm. per kilogram per day.

In order to distinguish whether this is a specific effect of the fluorid, or a mere "salt-action," control experiments were made by adding corresponding quantities of sodium chlorid to the food, instead of sodium fluorid. The food, with either drug,

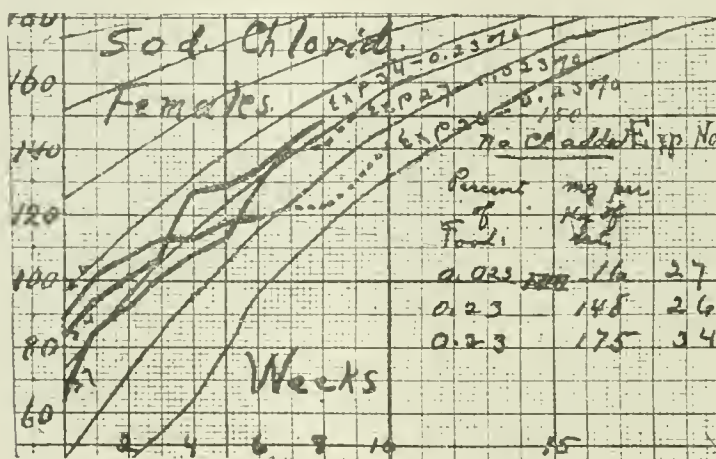


FIG. 7. SODIUM CHLORID ON GROWTH OF FEMALE RATS

already contained 1 per cent of NaCl. The quantities added in these experiments are in addition to this amount. To obtain the total concentration of NaCl, 1 per cent must be added to the first column of table 5.

Dosage of sodium chlorid. The quantities added to the standard food is shown in table 5. They duplicated the toxic range of the fluorid.

Effects of sodium chlorid on growth. These are shown in table 6 and figures 7 and 8. With 0.023 per cent of NaCl (experiment 27, fig. 7), the growth is normal during the seven weeks of drugged food and is not increased during the subsequent two weeks of unpoisoned food.

With 0.23 per cent, two series (34 and 47) show normal growth. In one series (26) growth was considerably checked (2.3 per cent per week) but the check was not nearly as great as with corre-

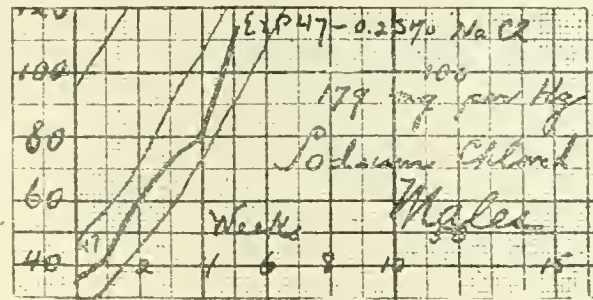


FIG. 8. SODIUM CHLORID ON GROWTH OF MALE RATS

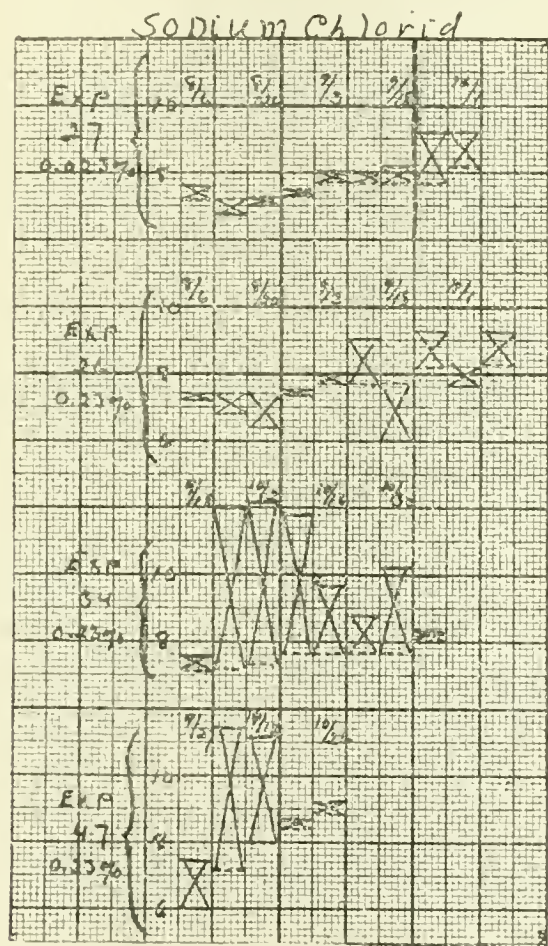


FIG. 9. SODIUM CHLORID ON FOOD CONSUMPTION

sponding doses of fluorid (6 to 9 per cent per week). This was accompanied by inadequate food-consumption. The growth recovered partly on unpoisoned food.

Effects of NaCl on food consumption. As shown in table 7 and figure 9 the animals of three series ate considerably more than the standard quantity of food; but with one exception the

TABLE 5
Dosage of sodium chlorid added to the standard food

NaCl ADDED TO THE STANDARD FOOD	EXPERIMENT NUMBER	NUMBER OF ANIMALS IN EXPERIMENTS	DURATION	MEAN DOSAGE, OF ADDED NaCl PER KILOGRAM OF RAT PER DAY
<i>per cent</i>			<i>weeks</i>	<i>mgm.</i>
0.023	27	5	7	16.1 (13-20.7)
0.23	26	5	6	148.0 (140-163)
0.23	34	5	8	175.0 (130-263)
0.23	47	4	5	179.0 (110-455)

TABLE 6
Effects of NaCl on growth

NaCl ADDED TO FOOD	MEAN ADDED DOSAGE PER KILOGRAM OF RAT PER DAY	EXPERIMENT NUMBER	DURATION	OBSERVED WEIGHT	NORMAL WEIGHT	DIFFERENCE	DIFFERENCE OF NORMAL WEIGHT	DIFFERENCE PER WEEK
<i>per cent</i>	<i>mgm.</i>		<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
0.023	16	27	7	140	135	+5	+3.6	+0.5
0.23	148	26	6	120	140	-20	-14.2	-2.3
0.23	175	34	8	148	150	-2	-1.3	-0.16
0.23	179	47	5	114	104	+10	+9.6	+1.9

TABLE 7
Sodium fluorid on food consumption

NaCl ADDED TO FOOD	EXPERIMENT NUMBER	DURATION OF EXPERIMENT	GROWTH, MEAN DIFFERENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMPTION MEAN DIFFERENCE FROM NORMAL STANDARD	
				Grams per rat per day	Per cent
<i>per cent</i>		<i>weeks</i>			
0.023	27	7	+0.5	+0.3	+4.0
0.23	26	6	-2.3	-0.2	-2.6
0.23	34	8	-0.16	+2.2	+29.0
0.23	47	5	+1.9	+1.4	+17.0

differences are within the normal range. The average excess is 14.5 per cent. The food consumption is not increased when the animals are changed to undrugged food (experiment 26).

The result is in marked contrast with the 0.23 concentration of sodium fluorid which showed a deficit of 44 per cent. The loss of appetite under fluorid is therefore certainly not due to mere "salt-action."

Mortality under sodium chlorid. Table 7 shows for 0.23 per cent NaCl a mortality of 3 animals out of 14, i.e., 21 per cent. The deaths are doubtless coincidences.

TABLE 8
Mortality

NaCl ADDED TO THE STANDARD FOOD	DOSAGE	EXPERIMENT NUMBER	NUMBER OF ANIMALS	WEEKS OF FATALITIES	TOTAL DURATION	FATALITIES
<i>per cent</i>					<i>weeks</i>	<i>per cent</i>
0.023	16	27	5	0	7	0
0.23	148	26	5	3	6	20
0.23	175	34	5	4	8	20
0.23	179	47	4	4	5	25

C. "PHOSPHATE ROCK"

The failure of sodium chlorid to produce the phenomena of sodium fluorid poisoning indicates clearly that the latter is not a "salt-action." Interesting confirmation of this is furnished by the feeding of "phosphate rock," i.e., calcium phosphate mineral, containing the fluorid probably as a difficultly soluble compound of calcium.

Dosage. The specimen of "phosphate rock" used in this series was obtained through the United States Bureau of Chemistry. It was reported to contain about 4 per cent of fluorin, equivalent to 9 per cent of sodium fluorid. For comparison it will be convenient to speak of the dosage in equivalents of sodium fluorid. This is shown in table 9.

Effects of phosphate rock on growth. These are shown in table 10 and figures 10 and 11. It will be noted that up to and including the daily dosage of 89 mgm. NaF or 0.92 gram of phosphate rock, per kilogram of rats, the growth is within the standard limits, and is not improved by changing to undrugged food. However, the growth is distinctly below the average standard,

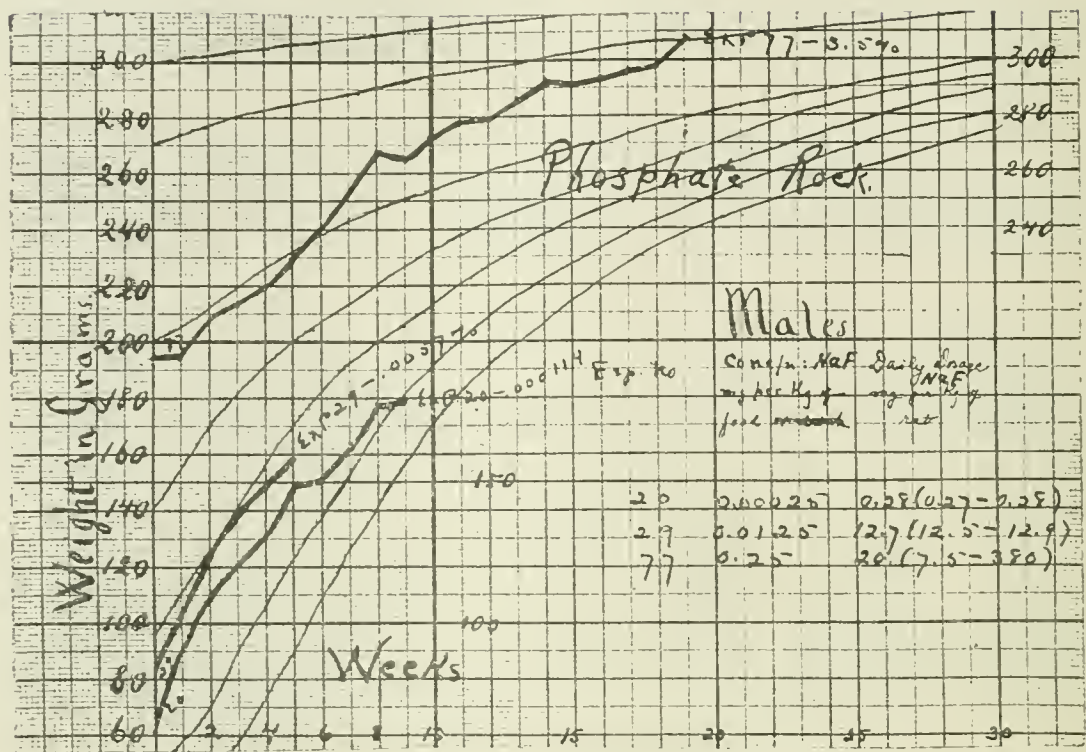


FIG. 10. PHOSPHATE ROCK ON GROWTH OF MALE RATS

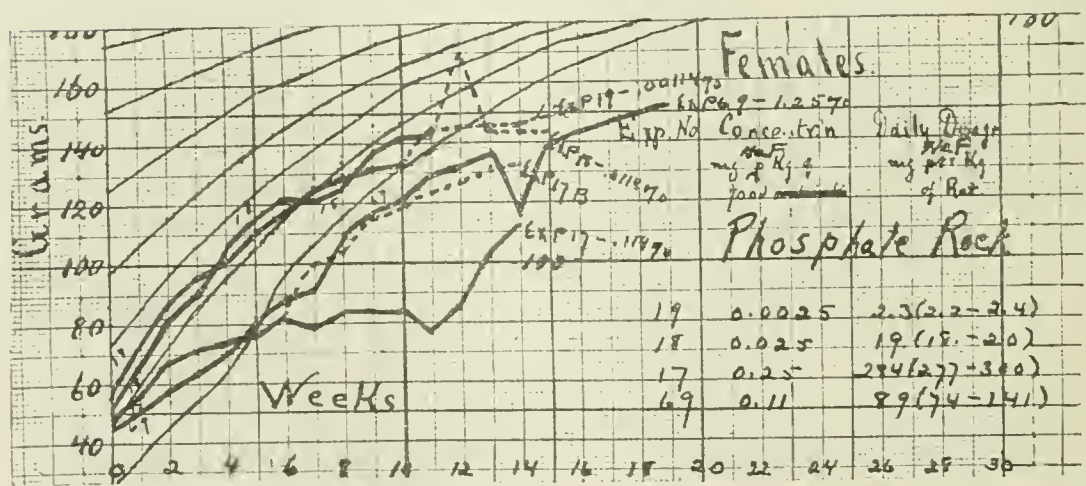


FIG. 11. PHOSPHATE ROCK ON GROWTH OF FEMALE RATS

with daily doses corresponding to 19 mgm. of NaF or 0.27 gram of phosphate rock and higher. A very distinct check of growth occurs in experiment 17 (fig. 11), with the mean daily dosage of 3.89 gram of rock or 284 mgm. of NaF per kilogram of rats.

TABLE 9
Dosage of phosphate rock

PHOS- PHATE ROCK IN FOOD	EQUIVA- LENT PERCENT- AGE OF SODIUM FLUORID	EXPERI- MENT NUM- BER	NUM- BER OF ANI- MALS IN EXPERI- MENTS	DURA- TION	MEAN DOSAGE OF DRUG PER KILOGRAM OF RAT PER DAY, IN TERMS OF	
					Rock	NaF
<i>per cent</i>				<i>weeks</i>	<i>mgm.</i>	<i>mgm.</i>
0.0035	0.00025	20	10	9	03.95 (3.81-4.09)	0.28 (0.27-0.28)
0.035	0.0025	19	10	10	33.00 (31-34)	2.30 (2.2-2.4)
0.17	0.0125	29	5	5	173.00 (170-175)	12.70 (12.5-12.4)
0.35	0.025	18	10	10	266.00 (252-280)	19.00 (18-20)
3.5*	0.25	77*	2	19	280.00 (105-3820)	20.00 (7.5-380)
1.25	0.11	69	6	20	920.00 (850-1612)	89.00 (74-141)
3.5	0.25	17	10	14	3980.00 (3885-4200)	284.00 (277-300)

* In this experiment, the rats had equal access to unpoisoned food.

TABLE 10
Effects of phosphate rock on growth

MEAN DOSE, PER KILOGRAM		EXPERI- MENT NUMBER	DURA- TION	OBSERVED WEIGHT	NORMAL WEIGHT	DIFFER- ENCE	DIFFER- ENCE OF NORMAL WEIGHT	DIFFER- ENCE PER WEEK
Rock	NaF							
<i>mgm.</i>	<i>mgm.</i>		<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
3.95	0.28	20	9	180	185	-5	-2.7	-0.3
33.0	2.3	19	10	134	150	-16	-1.0	-0.1
173.0	12.7	29	5	158	150	+8	+5.3	+1.0
266.0	19.0	18	10	140	148	-8	-5.4	-0.54
280.0	20.0	77	19	307	275	+32	+11.0	+0.57
920.0	89.0	69	19	152	180	-28	-15.6	-0.82
3980.0	284.0	17	11	77	146	-69	-46.0	-4.2
			14	112	160	-48	-30.0	-2.1

The growth recovers only partly when the animals are replaced on unpoisoned food.

It appears therefore that the "phosphate rock" checks the growth, analogous to sodium fluorid. A somewhat higher dosage of fluorin content is required in the case of the rock than

with the easily soluble fluorid; but the difference is not greater than 2 or 3 parts of the rock fluorid per 1 of sodium fluorid.

Effects of phosphate rock on food consumption. Table 11 and figure 12 show that the response is variable up to and including the daily dosage corresponding up to 98 mgm. of NaF or 0.92 gram of phosphate rock per kilogram; but there is a tendency for the food consumption to be subnormal with daily doses corresponding to 19 mgm. of NaF or 0.27 gram of phosphate rock per kilogram, and higher. With the daily dosage of 284 mgm. of NaF (3.89 grams of phosphate rock) the food consumption is checked very materially, i.e., 35 per cent below normal (experi-

TABLE 11
Phosphate rock on food consumption

MEAN DOSE, PER KILOGRAM		EXPERIMENT NUMBER	DURATION OF EXPERIMENT	GROWTH, MEAN DIFFER- ENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMPTION MEAN DIFFERENCE FROM NORMAL STANDARD	
Rock	NaF				Grams per rat per day	Per cent
<i>mgm.</i>	<i>mgm.</i>		<i>weeks</i>			
3.95	0.28	20	9	-0.3	+0.2	+2.2
33.0	2.3	19	10	-0.1	-0.55	-7.1
173.0	12.7	29	5	+1.0	+0.2	+2.2
266.0	19.0	18	10	-0.54	-0.8	-10.0
280.0	20.0	77	19	+0.57	+6.0	+53.0
920.0	89.0	69	20	-0.82	-0.6	-6.0
3980.0	284.0	17	14	-2.1	-2.65	-35.0

ment 17). This is about the same amount of reduction as when sodium fluorid was fed directly (151 mgm. NaF per day = 44 per cent reduction in food).

A "double cup" experiment (no. 77) was tried to determine whether the loss of appetite was due to the taste of the food. Figure 13 shows that both foods were eaten with equal avidity for six weeks; when a marked preference for the unpoisoned food or distaste for the poisoned food, developed. Evidently, the diminished food consumption in experiment 17 was not due to the direct taste of the food; but a discrimination against it must have been acquired in some other manner.

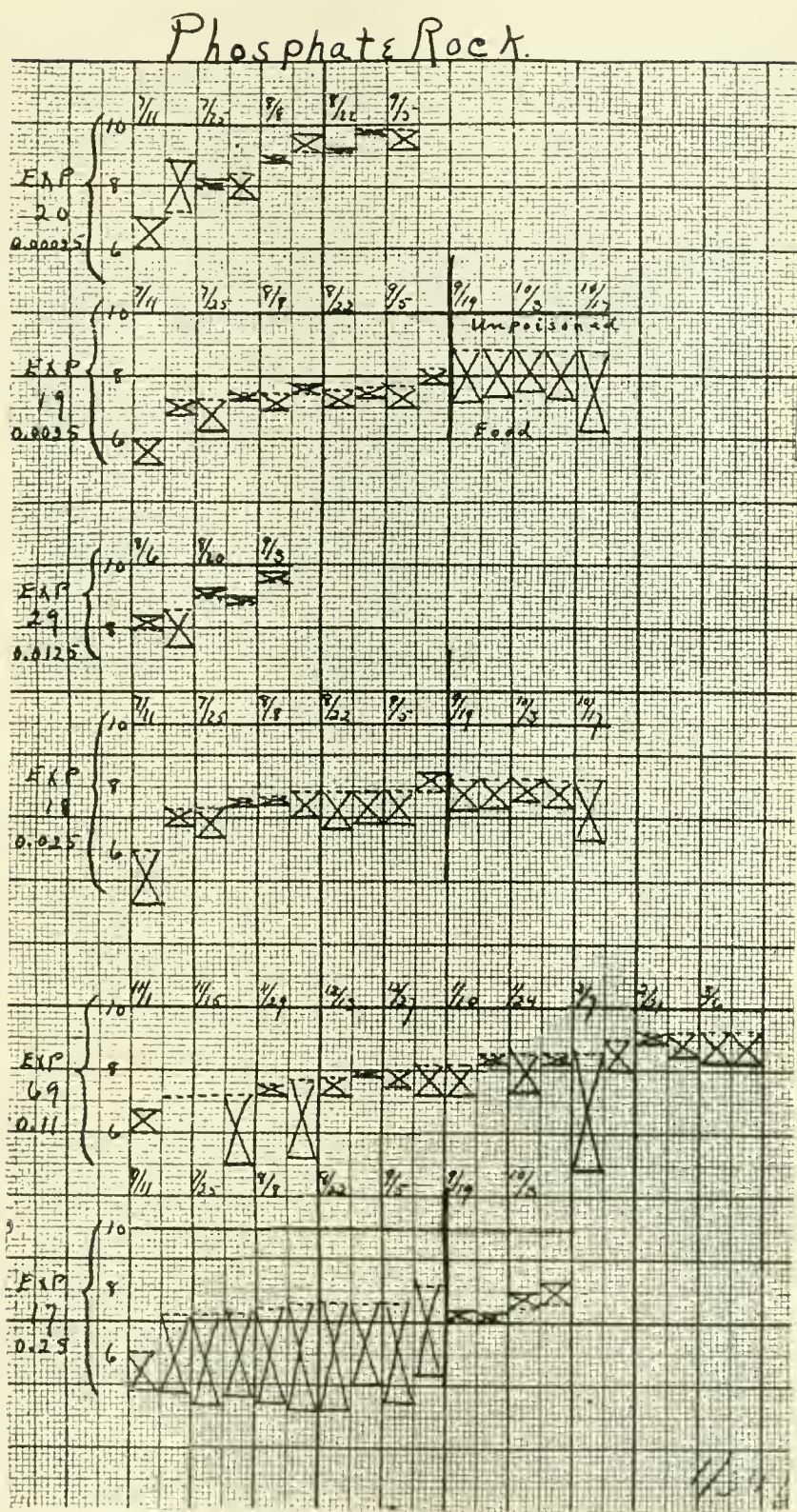


FIG. 12. PHOSPHATE ROCK ON FOOD CONSUMPTION

The rats of the two cup experiment 77, incidentally had a very abnormally high appetite at first, so much so that a technical error might be suspected, were it not that the growth curve is also unusual. Probably both were accidental, rendered pos-

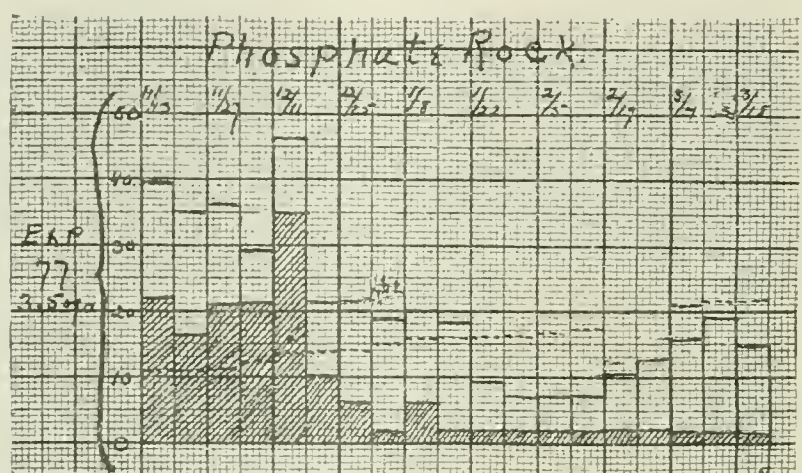


FIG. 13. CHOICE OF PHOSPHATE ROCK AND UNPOISONED FOOD

sible by the use of too small a number of rats (two only) in the experiment.

Mortality under phosphate rock. As shown in table 12, this is only 20 per cent with the largest dose; i.e., it is essentially negative.

TABLE 12
Mortality

MEAN DOSE, PER KILOGRAM		EXPERIMENT NUMBER	NUMBER OF ANIMALS	WEEKS OF FATALITIES	TOTAL DURATION	FATALITIES
Rock	NaF					
<i>mgm.</i>	<i>mgm.</i>				<i>weeks</i>	<i>per cent</i>
3.95	0.28	20	10	0	9	0
33.0	2.3	19	10	0	10	0
173.0	12.7	29	5	0	5	0
266.0	19.0	18	10	0	10	0
280.0	20.0	77	2	0	19	0
920.0	89.0	69	6	2	20	16
3980.0	284.0	17	10	12, 12	14	20

D. CALCIUM PHOSPHATE

The preceeding section showed that phosphate rock, in the concentration of 3.5 per cent or in daily doses of 3.98 gram per kilogram of rat checks very materially the food-consumption and growth of rats; and that indications of this unfavorable effect occur with the concentrations of 0.35 per cent or daily doses of 0.27 gram per kilogram. The effects agreed essentially with those of sodium fluorid of equivalent fluorin content. It

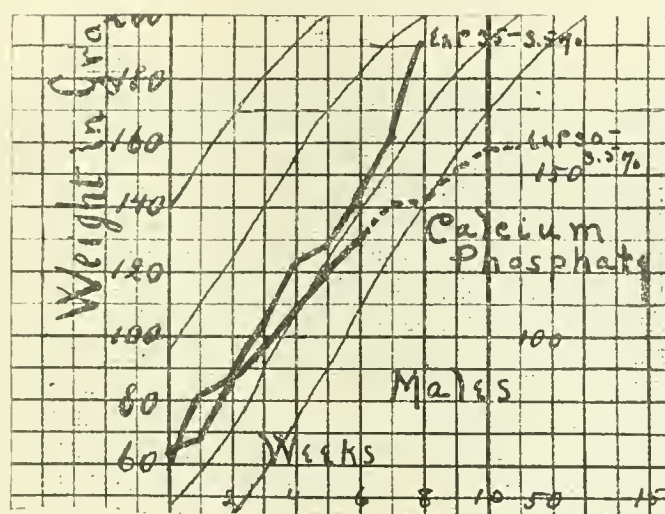


FIG. 14. CALCIUM PHOSPHATE ON GROWTH OF MALE RATS

seemed advisable, however, to determine whether ordinary pure ("precipitated") calcium phosphate in these doses has any effect on these functions. None was found.

Dosage of calcium phosphate. Three series of rats were fed with food to which 3.5 per cent of calcium phosphate had been added. This corresponds to the highest percent of "phosphate rock." The quantities consumed are shown in table 13.

Effects of calcium phosphate on growth. The data are contained in table 13 and figures 14 and 15. Only one series (experiment 30) showed retarded growth; and in this the retardation became even greater when undrugged food was substituted, so that the retardation of growth was evidently not due to the calcium phosphate.

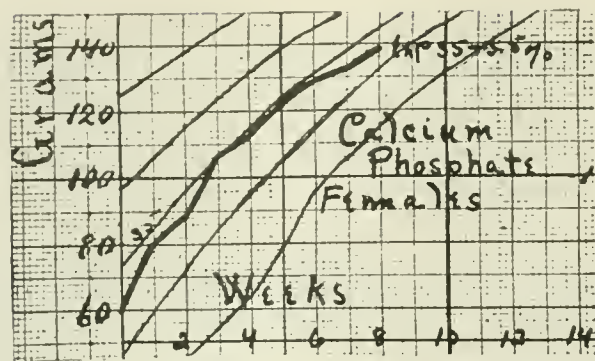


FIG. 15. CALCIUM PHOSPHATE ON GROWTH OF FEMALE RATS

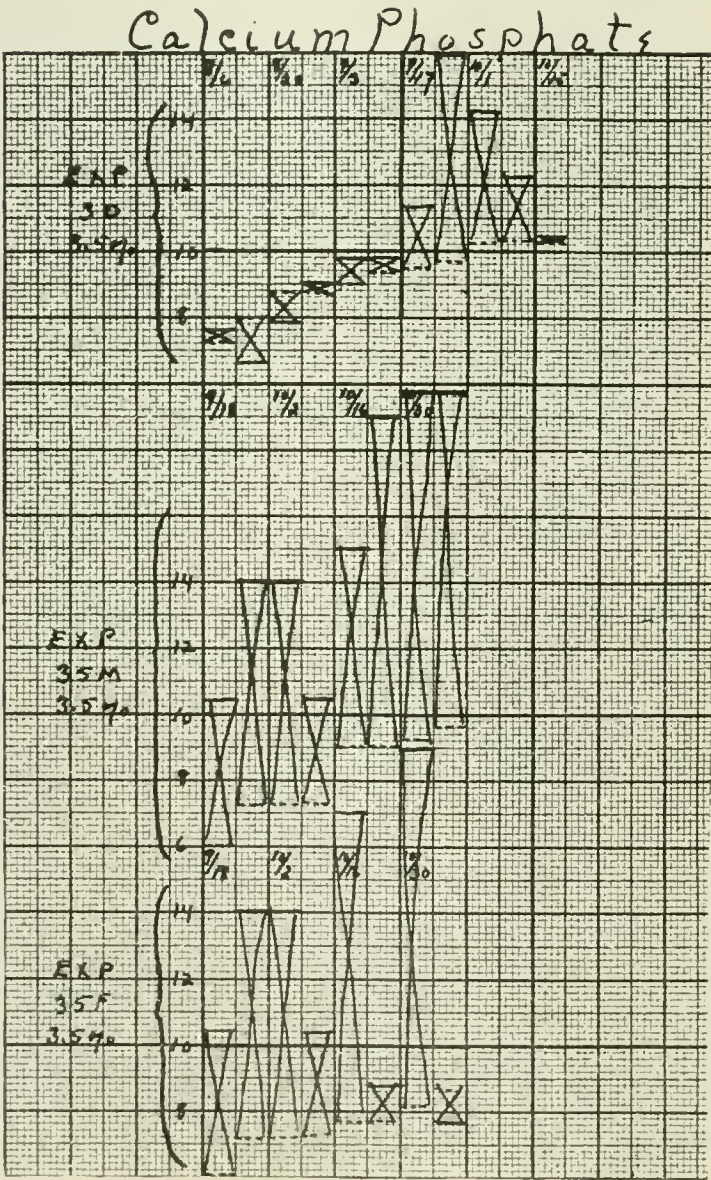


FIG. 16. CALCIUM PHOSPHATE ON FOOD CONSUMPTION

Effect of calcium phosphate on food consumption. This was normal or better in all experiments (table 14 and figure 16).

Calcium phosphate mortality. None of the animals died.

TABLE 13

Calcium phosphate on growth (3.5 per cent of the salt added to the food)

DOSAGE PER KILOGRAM OF RAT PER DAY	EXPERI- MENT NUMBER	NUMBER OF ANIMALS	DURA- TION	OB- SERVED WEIGHT	NORMAL WEIGHT	DIFFER- ENCE	DIFFER- ENCE OF NORMAL WEIGHT	DIFFER- ENCE PER WEEK
<i>grams</i>			<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
2.76 (2.55-3.88)	30	5	6	132	148	-16	-10.4	-1.7
4.14 (3.6-4.55)	35M	2	8	194	176	+18	+11.0	+1.4
3.50 (1.99-5.0)	35F	3	8	139	139	0	0	0

TABLE 14

Calcium phosphate (3.5 per cent) on food consumption

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	GROWTH, MEAN DIFFERENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMPTION, MEAN DIFFER- ENCE FROM NORMAL STANDARD	
			Grams per rat per day	Per cent
	<i>weeks</i>			
30	6	-1.7	+0.6	+6.8
35M	8	+1.4	+10.0	+109.0
35F	8	0	+9.7	+121.0

E. CALCIUM CARBONATE

It appeared interesting to determine whether the absence of effects observed with calcium phosphate applied also to other "insoluble" calcium salts. This was found to be the case. Three series of experiments were made with food to which 3.5 per cent of calcium carbonate was added. As the food already contains 1 per cent of calcium carbonate, the total percentage was 4.5.

Dosage. This is shown in table 15 in terms of the extra (3.5 per cent) calcium carbonate. It corresponds to the dosage of the phosphate.

Effects of calcium carbonate on growth. These are shown in table 15 and figures 17 and 18. Two of the series show normal growth or better. In series 69 there is an apparent check of

TABLE 15

Calcium carbonate on growth (3.5 per cent of the salt added to the standard food)

DOSAGE OF EXTRA CaCO ₃ PER RAT PER DAY	EXPERI- MENT NUMBER	NUMBER OF ANIMALS	DURA- TION	OB- SERVED WEIGHT	NORMAL WEIGHT	DIFFER- ENCE	DIFFER- ENCE OF NORMAL WEIGHT	DIFFER- ENCE PER WEEK
<i>grams</i>			<i>weeks</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
2.59 (2.34-3.36)	126	3	7	152	145	+7	+4.8	+0.68
3.56 (2.45-9.13)	65	2	9	138	185	-47	-25.0	-2.7
3.76 (3.18-5.88)	104	3	4	124	90	+34	+37.0	+9.2

TABLE 16

Calcium carbonate (extra 3.5 per cent) on food consumption

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	GROWTH, MEAN DIFFERENCE FROM NORMAL STANDARD PER CENT PER WEEK	FOOD CONSUMPTION, MEAN DIFFER- ENCE FROM NORMAL STANDARD	
			Grams per rat per day	Per cent
	<i>weeks</i>			
126	7	+0.68	+2.8	+37
65	9	-2.7	+2.25	+38
104	4	+9.2	+3.4	+33

growth, notwithstanding that the animals had good appetite. The growth curve, however, is so irregular that the series may properly be disregarded.

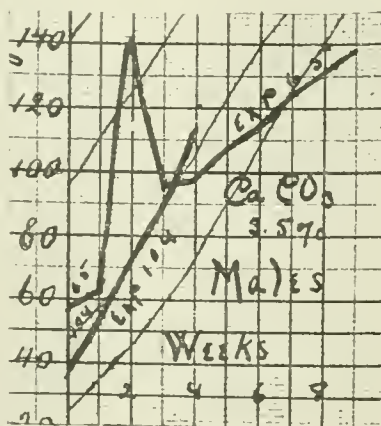


FIG. 17. CALCIUM CARBONATE ON GROWTH OF MALE RATS

Calcium carbonate effects on food consumption. As shown in table 16 and figure 19, the food consumption in all of the animals was better than standard.

Calcium carbonate mortality. None of the animals died.

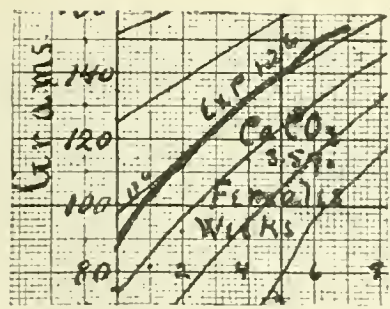


FIG. 18. CALCIUM CARBONATE ON GROWTH OF FEMALE RATS

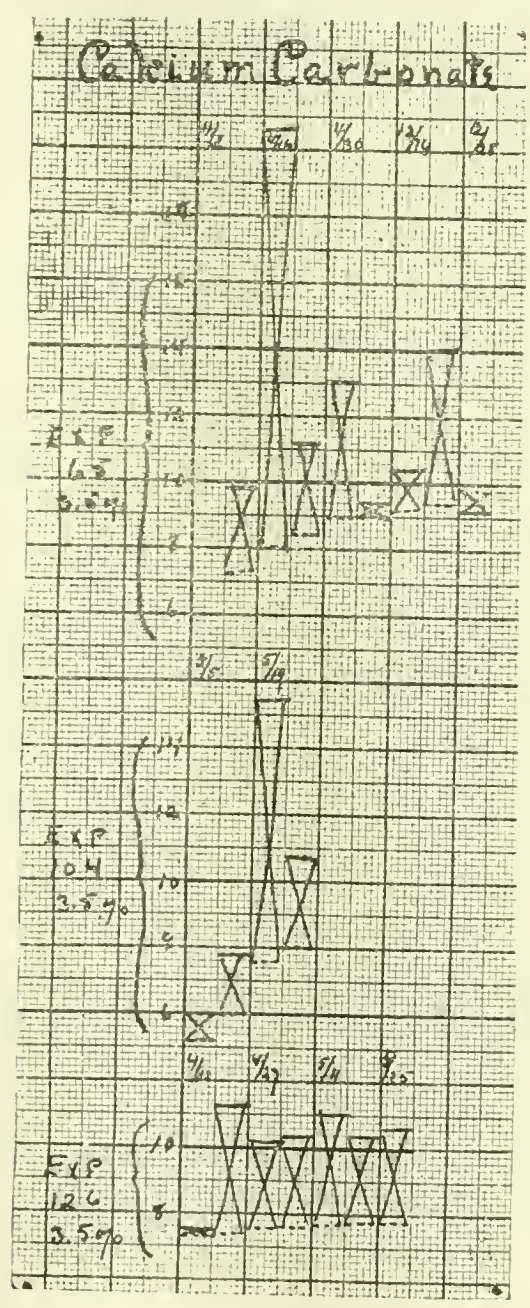


FIG. 19. CALCIUM CARBONATE ON FOOD CONSUMPTION

SUMMARY

A. *Sodium fluorid.* This was administered to rats in their food, for continuous periods up to twenty-one weeks: followed in some cases by after-periods of unpoisoned food. The effects differ quantitatively according to the daily dosage.

Daily doses of 42 to 151 mgm. of NaF per kilogram of rats produced very marked retardation of growth, starting with the administration, and increasing with the dosage. The damage is persistent, i.e., if the administration of the drug is stopped, growth returns toward normal; but not sufficiently to make up materially in twelve weeks, the loss that occurred during the drug period.

The food-consumption is generally diminished, parallel to the growth. This is not due to distaste for the food; for except with the highest concentration, the rats did not discriminate against the poisoned food, when permitted equal access to unpoisoned food.

The highest of these fluorid doses (151 mgm.) is uniformly fatal within eleven weeks. Doses of 87 mgm. or smaller, for long periods, apparently are not fatal; for the mortality of the animals was small, no greater than that of control animals.

Daily doses of 15 to 20 mgm. of NaF per kilogram of rats produced after a time moderate but definite interference with the growth and food consumption. This effect may occur early, or be delayed for fifteen weeks. Recovery of normal weight on unpoisoned food is slow and imperfect.

Daily doses of 0.15 to 8 mgm. of NaF per kilogram of rats for nine weeks did not perceptively affect growth or food consumption.

B. *Sodium chlorid.* Concentrations of NaCl equivalent to the highest concentrations of NaF, when added to the standard food, do not diminish the growth or food consumption. The latter indeed appears larger than usual; but this may be accidental.

C. "*Phosphate rock.*" Fluorid in this form has approximately the same deleterious effects as corresponding doses in the form of NaF.

D and E. Calcium phosphate or calcium carbonate. In doses corresponding to those of "phosphate rock," these do not depress growth or appetite. Indeed, the food consumption was generally much better than usual; but this again might have been accidental.

CONCLUSIONS

Sodium fluorid eaten with food during long periods in daily doses of 15 to 150 mgm. per kilogram of body weight, results in progressive impairment of growth and food consumption.

The damage is proportional to the dose. It tends to outlast the administration of the drug; so that the growth of animals that have been poisoned with fluorid remains permanently below that of unpoisoned animals.

The diminished food consumption is not due to distaste of the fluorid food; for the animals do not prefer unpoisoned food (except with the highest concentrations).

The effects are not due to "general salt action" for corresponding doses of NaCl are not harmful.

Fluorid in the form of "phosphate rock" produces the same effects as NaF, and in nearly corresponding doses.

The deleterious effect of phosphate rock is not shared by other insoluble Ca salts (phosphate and carbonate) in equal concentration.

No deleterious effects on growth or food consumption occurred in nine weeks with daily doses of NaF of 8 mgm. per kilogram of body weight, or with smaller doses.

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THE ACTION OF DRUGS UPON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS VII. PHYSOSTIGMINE

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The experiments were made on cats. The animals were anesthetised with ether. Blood was collected from the adrenals in the usual way and its concentration in epinephrin assayed on rabbit intestine (and uterus) segments. Of course, indifferent blood obtained after injection of the drug was used for the assay of the subsequent adrenal specimens.¹ Usually the assay of the specimen collected after physostigmine was made both on the non-atropinised segment and on the segment after it had been atropinised. Although atropin generally increases the sensitiveness of an intestine segment to epinephrin, thus sometimes permitting a sharper assay, the concentration determined for a given specimen is essentially the same as with the non-atropinised segment. It may be further remarked here, as a small point in

¹To control the possibility that the indifferent blood collected at the end of the experiment might contain an appreciably smaller quantity of any of the drugs studied than the adrenal blood specimen collected soon after administration of the drug, and that the inhibition of the intestine segment which was depended upon for the estimation of the epinephrin content might thus be influenced by the drug, indifferent blood to which the drug was added in the maximum amount which could have been present immediately after its injection was also employed, without altering the result of the assays. In any case it would be quite impossible to explain such a phenomenon as the abrupt change of sign of the nicotine effect (4) (the great augmentation of the output for the first 30 or 45 seconds, rapidly passing into a marked and long lasting diminution) on the basis of a difference in the concentration of the drug in the successive specimens as compared with its concentration in the indifferent blood used for the assay.

technique, that a segment which has been contracting with some irregularity and cannot therefore yield very good results in assaying epinephrin, is not infrequently markedly improved in this regard by atropinisation. This suggests that the local nervous mechanism is responsible for the irregularity. This hypothesis would also explain the fact that a segment which has not been satisfactory at the beginning may greatly improve in constancy of response to epinephrin and in regularity of contraction after it has been worked with for some hours, during which time, it may be supposed, the local nervous mechanism has become inactive.

Except in experiments made for the purpose of defining the seat of the action, the blood supply of the stomach and intestines was not interfered with. In these experiments the coeliac axis and superior mesenteric artery were usually tied in order to prevent too great a fall of blood pressure when the nerve supply of the adrenals, including the splanchnics, was cut. The dose varied from 0.036 mgm. to 1.5 mgm. of physostigmine sulphate per kilogram of body weight, administered intravenously and from 0.23 mgm. to 1.6 mgm. per kilogram of body weight administered subcutaneously.

The results of the experiments were in sharp contrast to those on pilocarpine previously reported (1). They showed consistently an increased epinephrin output up to as much as 10 or 15 times the initial output, an increase of the same order of magnitude as that produced by strychnine (2). This increase does not develop immediately. Indeed, a specimen of adrenal vein blood collected within a minute or two after the intravenous injection of physostigmine usually shows a marked diminution in the rate of output, which then increases and may not reach its maximum for a considerable time. The largest outputs were usually observed in specimens collected 20 to 30 minutes after the administration of the drug. But we do not know whether still more remote specimens might not show a still larger output. It is at any rate clear that, as in the case of strychnine, the action is by no means a transient one. An apparent difference between the physostigmine and the strychnine action is that we have repeatedly observed with physostigmine that the concentration

of epinephrin can be driven up far beyond anything, seen in normal adrenal vein blood (to as much as 1:150,000 in the serum).

Specimens of the protocols and of the tracings used in the assay follow.

EXPERIMENTS WITH INTRAVENOUS INJECTION OF PHYSOSTIGMINE

Condensed protocol. Cat 468; female (pregnant); weight 3.42 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein. Obtained indifferent (jugular) blood.

9.45 a.m. Cava pocket completed; collected adrenal blood.

9.45½ a.m. First specimen, 1.8 grams in 30 seconds (3.6 grams per minute).

9.46 a.m. Second specimen, 4.7 grams in 90 seconds (3.13 grams per minute). Blood pressure, 132 mm.² of mercury.

9.49½ a.m. End of intravenous injection of 2 mgm. of physostigmine sulphate. Blood pressure, 112 mm. of mercury, rising quickly to 140 mm.

9.50 a.m. Third specimen, 3.95 grams in 1 minute.

9.51 a.m. Fourth specimen, 5.8 grams in 3 minutes (1.93 grams per minute). Blood pressure, 85 mm. of mercury; muscular twitching, profuse salivation and lachrymation, sweating in pads. Spontaneous respiration good; occasional gasps; started artificial respiration and continued it throughout rest of experiment. Pupils at first dilated, later constricted and mictitating membranes forward.

10.15 a.m. Blood pressure 89 mm. of mercury, above symptoms more marked.

10.15½ a.m. Fifth specimen, 1.05 gram in 30 seconds, (2.1 grams per minute).

10.16 a.m. Sixth specimen, 5 grams in 3 minutes, (1.7 grams per minute). Blood pressure 74 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.562 gram.

² The blood pressures given in the protocols are always those at the end of collection of the specimens, and are usually somewhat lower than the pressures at the beginning of collection.

In cat 468 the dose of physostigmine was 0.6 mgm. per kilogram. The second adrenal specimen (collected before administration of the drug) was decidedly weaker than 1:3,750,000 adrenalin, stronger than 1:7,500,000, weaker than 1:5,000,000, somewhat stronger than 1:6,250,000. It was taken at 1:6,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute. The third specimen, collection of which was begun 30 seconds after the end of injection of the physostigmine, caused no inhibition of the segment till it was atropinised, after which it gave a small reaction. The fourth specimen was much stronger than the third, and the sixth specimen (collected 27 minutes after the drug was given) was very much stronger than the fourth. The fourth specimen was stronger than 1:5,000,000, decidedly weaker than 1:2,500,000, somewhat stronger than 1:3,750,000 (fig. 1). It was finally taken at 1:3,500,000, corresponding to an output of 0.00055 mgm. per minute for the cat, or 0.00016 mgm. per kilogram per minute, the same as the initial output. The sixth specimen was found to be weaker than 1:500,000, weaker than 1:625,000, and decidedly stronger than 1:930,000 (fig. 2). It was taken at 1:700,000, giving 0.0024 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute, 5 times the initial output.

In the next experiment (cat 469) the initial output was nearly double what it was in cat 468. It was nevertheless decidedly increased under the influence of a dose of physostigmine of 1.5 mgm. per kilogram of body-weight.

Specimens of the tracings are given in figures 3 to 5.

Condensed protocol. Cat 469; female; weight, 3.3 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein; cut both vago-sympathetics. Obtained indifferent (jugular) blood.

9.38 a.m. Cava pocket completed. Collected adrenal blood.

2.39½ a.m. First specimen, 2 grams in 30 seconds (4 grams per minute).

9.40 a.m. Second specimen, 6.6 grams in 90 seconds (4.4 grams per minute). Blood pressure, 103 mm. of mercury.

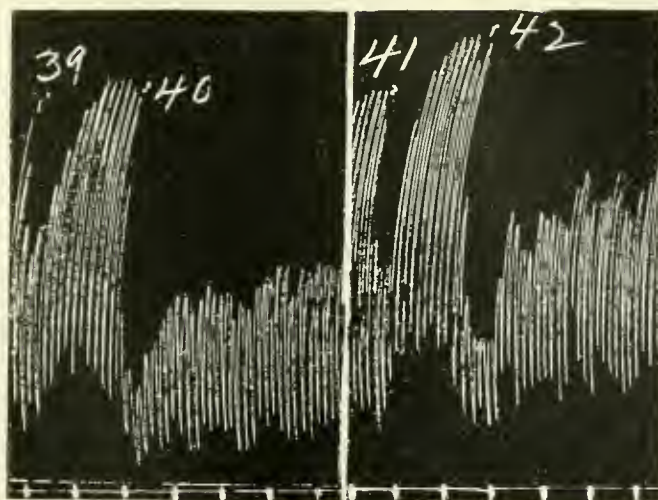


FIG. 1. INTESTINE TRACINGS. BLOODS FROM CAT 46S

At 39 and 41 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 40 by the 4th adrenal blood specimen (collected $1\frac{1}{2}$ minutes after injection of physostigmine); at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:3,750,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). In all figures the time trace is in half-minutes. Reduced to two-thirds.

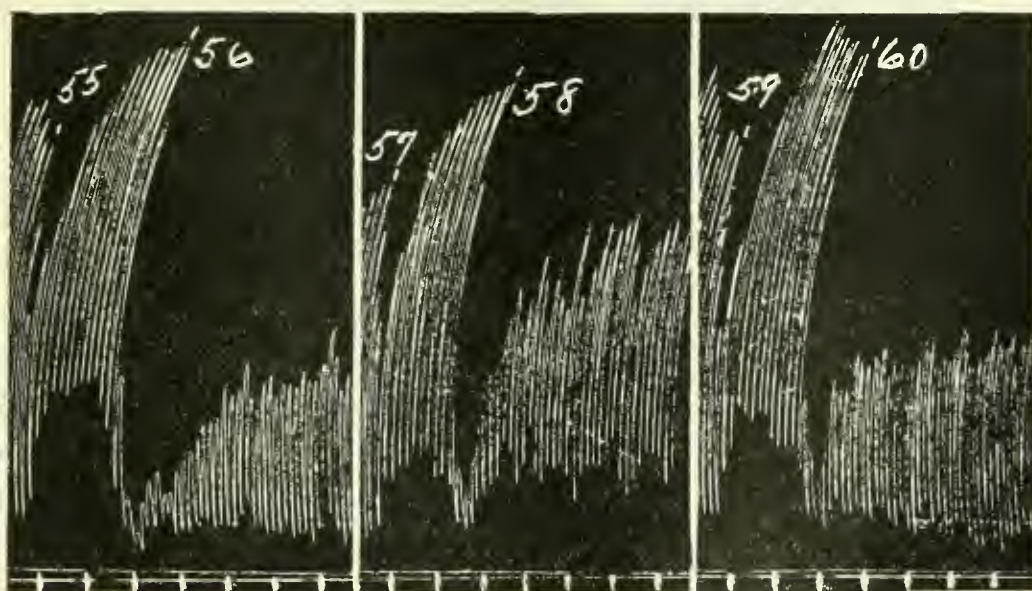


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT 46S

At 55, 57, and 59 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 56 by indifferent blood to which was added adrenalin to make a concentration of 1:625,000; at 58 by indifferent blood to which was added adrenalin to make a concentration of 1:930,000; at 60 by the 6th adrenal blood specimen (collected $26\frac{1}{2}$ minutes after injection of physostigmine). All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

- 9.46 $\frac{1}{2}$ a.m. End of intravenous injection of 5 mgm. physostigmine sulphate. Blood pressure 92 mm. of mercury. Spontaneous respiration ceased temporarily. Started artificial respiration and continued it throughout rest of experiment.
- 9.47 a.m. Third specimen, 2.85 grams in 1 minute.
- 9.48 a.m. Fourth specimen, 5.05 grams in 3 minutes (1.7 grams per minute). Blood pressure, 52 mm. of mercury. Pupils became contracted; profuse salivation, lachrymation and sweating (in pads); muscular twitching began and became more marked as the experiment progressed.

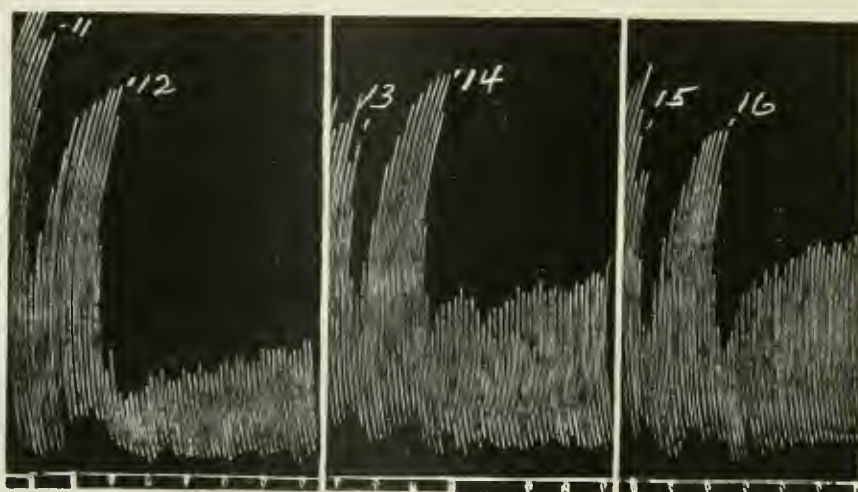


FIG. 3. INTESTINE TRACINGS. BLOODS FROM CAT 469

At 11, 13, and 15 Ringer was replaced by indifferent blood and this at 12 by indifferent blood to which was added adrenalin to make a concentration of 1:3,570,000; at 14 by the 2d adrenal blood specimen (collected before injection of physostigmine); at 16 by indifferent blood to which was added adrenalin to make a concentration of 1:5,700,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

- 10.12 a.m. Above symptoms more marked. Blood pressure 52 mm. of mercury. Spontaneous respirations present.
- 10.14 a.m. Fifth specimen (not weighed).
- 10.14 $\frac{1}{2}$ a.m. Sixth specimen, 3.6 grams in 5 minutes (0.72 gram per minute). Blood pressure, 36 mm. of mercury.
- Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.399 gram.

The second specimen (collected before injection of physostigmine) was found to be much stronger than 1:8,300,000, stronger than 1:5,700,000, decidedly weaker than 1:3,570,000 (fig. 3). It

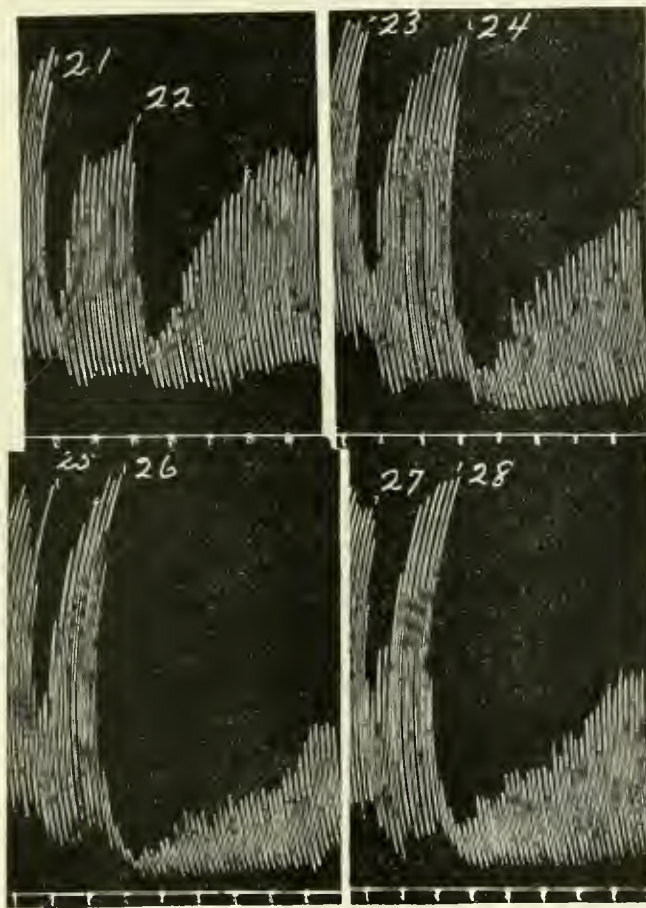


FIG. 4. INTESTINE TRACINGS. BLOODS FROM CAT 469

At 21, 23, 25 and 27 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 22 by the 3d adrenal blood specimen (collected $\frac{3}{4}$ minute after injection of physostigmine); at 24 by the 4th adrenal blood specimen (collected $1\frac{3}{4}$ minutes after injection of physostigmine); at 26 by the 6th adrenal blood specimen (collected 28 minutes after injection of physostigmine) diluted with 2 volumes indifferent blood; at 28 by the 6th adrenal blood specimen diluted with 4 volumes indifferent blood. All the bloods were diluted with 3 volumes Ringer (the 6th specimen after diluting with indifferent blood). Reduced to one-half.

was assayed at 1:5,000,000, corresponding to an output of 0.00088 mgm. per minute for the cat, or 0.00026 mgm. per kilogram per minute. The third specimen, obtained 45 seconds after administration of the drug, was weaker than the fourth, collected one

minute later, and much weaker than the sixth specimen (collected about half an hour after the injection), even when the latter was diluted with 4 volumes of indifferent blood (fig. 4). The third specimen was stronger than 1:5,700,000, weaker than 1:4,300,000 and was assayed at 1:5,000,000, corresponding to an output of 0.00057 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

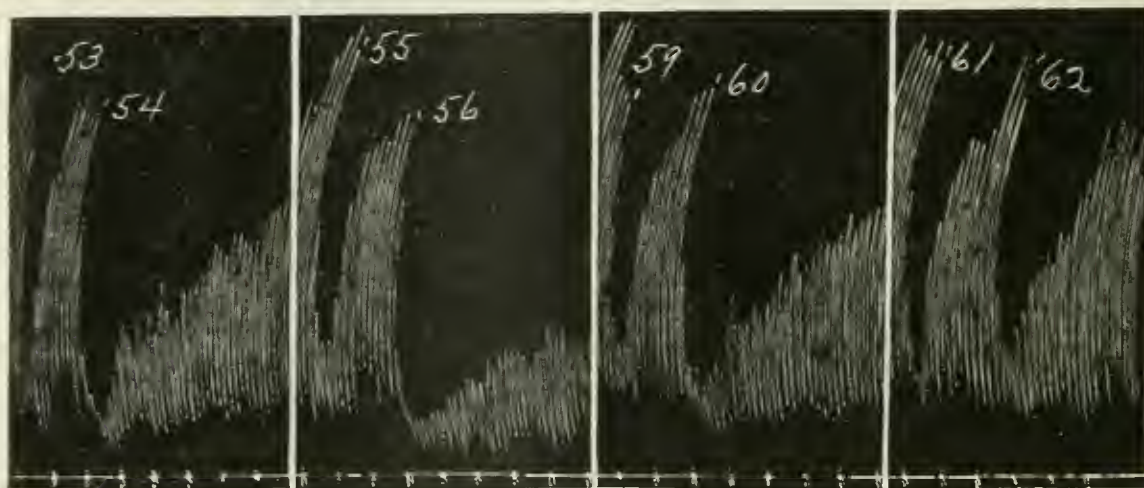


FIG. 5. INTESTINE TRACINGS. BLOODS FROM CAT 469

At 53, 55, 59 and 61 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 54 and 60 by the 4th adrenal blood specimen (collected 1½ minutes after injection of physostigmine) diluted with 1 volume indifferent blood; at 56 by indifferent blood to which was added adrenalin to make a concentration of 1:2,800,000; at 62 by indifferent blood to which was added adrenalin to make a concentration of 1:5,700,000. All the bloods were diluted with 3 volumes Ringer (the adrenal blood after diluting with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

The fourth specimen was weaker than 1:1,400,000, decidedly stronger than 1:2,850,000 (fig. 5). It was assayed at 1:1,600,000, giving an output of 0.001 mgm. per minute for the cat, or 0.0003 mgm. per kilogram per minute. The sixth specimen, diluted with 4 volumes of the indifferent blood collected at the end of the experiment, had about the same epinephrin concentration as the four specimen undiluted with indifferent blood. The sixth was shown to be stronger than 1:570,000, decidedly weaker than

1:200,000, not much different from 1:285,000 but somewhat weaker. It was taken at 1:300,000 corresponding to an output of 0.0024 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute, nearly three times the initial output. It must be noted that the blood flow at the time of collection of the sixth specimen was only one-sixth as great as at the time of collection of the second. With the high concentration already reached, it could not be expected that the calculated output should show the full effect of the physostigmine excitation on the epinephrin secretory apparatus.

In cat 472 a much smaller dose of physostigmine, 0.17 mgm. per kilogram was employed. Yet the output of epinephrin was increased to 8 or 9 times the initial value, which was distinctly less than in cat 469. Here the blood flow remained good at the time of collection of the last specimen.

Condensed protocol. Cat 472; male; weight, 2.91 kgm.

Under ether, inserted cannulae into trachea, carotid artery, and external jugular vein. Cut both vago-sympathetics. Obtained indifferent (jugular) blood.

9.50 a.m. Cava pocket completed. Collected adrenal blood.

9.52½ a.m. First specimen, 1.4 grams in 30 seconds (2.8 grams per minute).

9.53 a.m. Second specimen, 4.4 grams in 2 minutes (2.2 grams per minute). Blood pressure 80 mm. of mercury.

10.04½ a.m. End of intravenous injection of 0.5 mgm. physostigmine sulphate. Blood pressure 84 mm. of mercury.

10.05 a.m. Third specimen, 3.55 grams in 1 minute. Blood pressure 99 mm. of mercury.

10.06 a.m. Fourth specimen, 5.2 grams in 2 minutes (2.6 grams per minute). Blood pressure 72 mm. of mercury. Muscular twitching beginning and slight increase in flow of saliva and tears. Pupils wide and mictitating membranes forward.

10.15 a.m. Respiration becoming shallow. Started artificial respiration. Sweating of pads present.

10.29 a.m. Above symptoms more marked. Blood pressure 68 mm. of mercury.

10.29½ a.m. Fifth specimen, 1.35 grams in 30 seconds (2.7 grams per minute).

10.30 a.m. Sixth specimen, 4.65 grams in 3 minutes (1.55 grams per minute). Blood pressure 50 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.368 gram.

The second specimen, obtained before administration of the drug, was found to be decidedly weaker than 1:3,750,000 adrenalin, stronger than 1:7,500,000, somewhat stronger than 1:6,250,000, not much different from 1:5,000,000. Taking it at

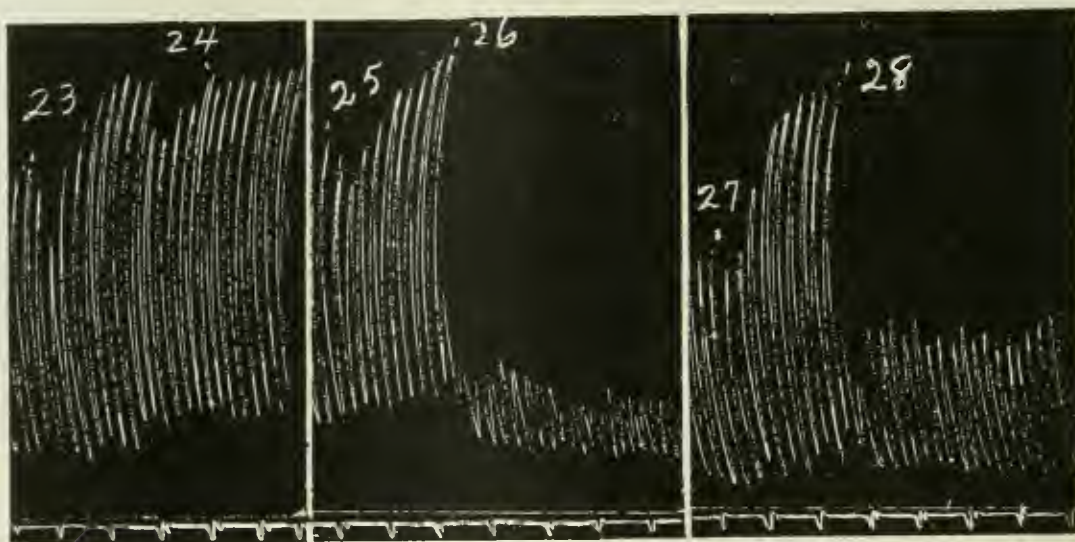


FIG. 6. INTESTINE TRACINGS. BLOODS FROM CAT 472

At 23, 25, and 27 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 24 by the 4th adrenal blood specimen (collected 1½ minutes after injection of physostigmine); at 26 by the 5th adrenal blood specimen (collected 25 minutes after injection of physostigmine); at 28 by the 6th adrenal blood specimen (collected 25½ minutes after injection of physostigmine) diluted with 3 volumes indifferent blood. All the bloods were diluted with 3 volumes Ringer (the 6th specimen after diluting with indifferent blood). Reduced to two-thirds.

1:5,000,000 we get 0.00044 mgm. per minute as the output of epinephrin for the cat, or 0.00015 mgm. per kilogram per minute.

Both the third and fourth specimens, collected immediately after the injection of physostigmine, had a very low concentration of epinephrin, much less than that of the fifth and sixth

specimens, collected 25 minutes later (fig. 6), although the adrenal blood flow was the same when the fourth and fifth specimens were obtained. The concentration of the third and fourth specimens was about the same, approximately 1:25,000,000, corresponding to an output of 0.00003 mgm. per kilogram per minute for the fourth specimen, only one-fifth of the initial rate.

The sixth specimen was decidedly stronger than 1:940,000, stronger than 1:625,000, somewhat weaker than 1:300,000,

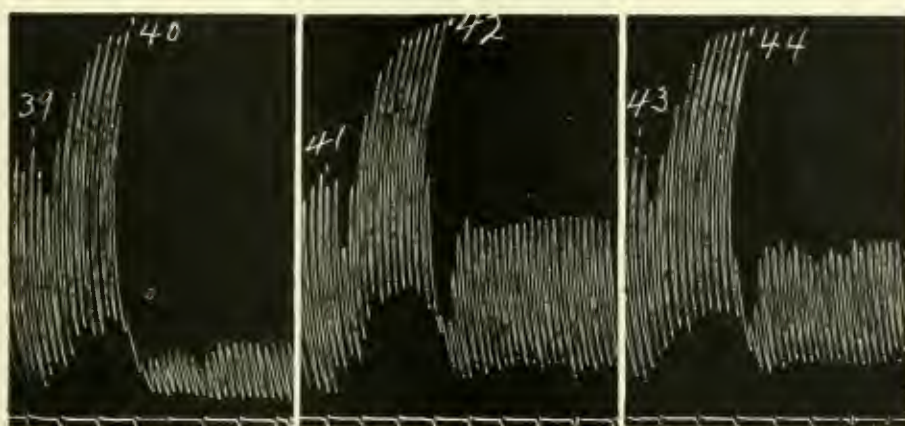


FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 472

At 39, 41, and 43 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 40 by indifferent blood to which was added adrenalin to make a concentration of 1:940,000; at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:1,880,000; at 44 by the 6th adrenal blood specimen (collected 25½ minutes after injection of physostigmine) diluted with 3 volumes indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenal blood after diluting with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

decidedly weaker than 1:245,000 and somewhat stronger than 1:470,000 (fig. 7). It was assayed at 1:400,000, corresponding to an output of 0.004 mgm. per minute for the cat, or 0.0014 mgm. per kilogram per minute, nine times the initial output.

No attempt was made to determine the smallest dose of physostigmine which would produce a definite effect. The smallest dose employed was 0.036 mgm. per kilogram (in cat 484).

Condensed protocol. Cat 484, female; weight 2.8 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein. Obtained indifferent (jugular) blood.

10.30 a.m. Cava pocket completed. Collected adrenal blood.

10.32 a.m. First specimen, 1.45 grams in 40 seconds (2.2 grams per minute).

10.32 $\frac{2}{3}$ a.m. Second specimen, 3.75 grams in 2 minutes (1.87 grams per minute). Blood pressure 80 mm. of mercury.

10.44 a.m. End of intravenous injection of 0.1 mgm. physostigmine sulphate. Blood pressure 106 mm. of mercury.

10.45 $\frac{1}{2}$ a.m. Third specimen, 1.05 gram in 30 seconds (2.1 grams per minute).

10.46 a.m. Fourth specimen, 4.05 grams in 2 minutes (2.02 grams per minute). Blood pressure 104 mm. of mercury.

10.55 a.m. Moderate salivation and lachrymation and muscular twitching present. No sweating of pads.

11.07 $\frac{1}{2}$ a.m. Fifth specimen, 0.8 gram in 30 seconds (1.6 grams per minute).

11.08 a.m. Sixth specimen, 3.92 grams in 2 minutes (1.96 grams per minute). Blood pressure 88 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.313 gram.

The changes in the output of epinephrin, although smaller than with larger doses, were distinct. This applies both to the preliminary diminution and to the subsequent augmentation. The second specimen, collected before the drug was given, was decidedly stronger than 1:6,250,000 adrenalin, decidedly weaker than 1:2,500,000, weaker than 1:3,750,000, somewhat stronger than 1:5,000,000. It was taken at 1:4,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute. The fourth specimen, obtained 2 minutes after the injection of the drug, was much weaker than 1:6,250,000, weaker than 1:12,500,000 (confirmed by two sets of observations), much stronger than 1:25,000,000. It was taken at 1:15,000,000, corresponding to an output of 0.00013 mgm. per minute for the cat or 0.000045 mgm. per kilogram per minute, less than one-third of the initial output. The sixth specimen,

collected 24 minutes after the injection of physostigmine, was much stronger than the fourth, although the blood flows were about the same. It was decidedly stronger than 1:5,000,000, somewhat stronger than 1:3,750,000, weaker than 1:2,500,000. It was assayed at 1:3,000,000 giving an output of 0.00065 mgm. per minute for the cat, or 0.00023 mgm. per kilogram per minute, not quite double the initial output.

EXPERIMENTS WITH SUBCUTANEOUS INJECTION OF PHYSOSTIGMINE

Condensed protocol. Cat 471; female (pregnant); weight, 2.75 kgm.

Under ether inserted cannulae into traches, carotid artery and external jugular vein. Cut both vago-sympathetics. Obtained indifferent (jugular) blood.

9.35 a.m. Cava pocket completed. Collected adrenal blood.

9.38½ a.m. First specimen, 2.9 grams in 30 seconds (5.8 grams per minute).

9.39 a.m. Second specimen, 5.37 grams in 1 minute. Blood pressure 96 mm. of mercury.

9.43 a.m. End of subcutaneous injection of 3 mgm. physostigmine sulphate. Blood pressure 94 mm. of mercury.

9.47 a.m. Third specimen, 2.8 grams in 30 seconds (5.6 grams per minute). Blood pressure 104 mm. of mercury.

9.47½ a.m. Fourth specimen, 4.7 grams in 1 minute. Blood pressure 90 mm. of mercury.

9.52 a.m. Muscular twitching but no salivation or lachrymation. Respiration getting shallow; started artificial respiration. Blood pressure 75 mm. of mercury.

10.00 a.m. Marked increase in salivation, lachrymation and muscular twitching. No change in pupils (about two-thirds to three-quarters dilated). Blood pressure 62 mm. of mercury.

10.15 a.m. Above symptoms more marked and pupils constricted.

10.16½ a.m. Fifth specimen (not weighed).

10.17 a.m. Sixth specimen, 4.15 grams in 4 minutes (1.04 grams per minute). Blood pressure 43 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.41 gram.

In cat 471 a dose of 1.1 mgm. of the drug per kilogram of body-weight was injected subcutaneously. The second adrenal specimen, collected before administration of physostigmine, was decidedly weaker than 1:12,500,000 adrenalin (confirmed by several observations), somewhat stronger than 1:25,000,000, somewhat weaker than 1:18,750,000. It was finally taken at 1:20,000,000, corresponding to an output of 0.00027 mgm. per minute for the cat, or 0.0001 mgm. per kilogram per minute. The fourth specimen, collected 5 minutes after injection of the drug, was stronger than 1:6,250,000, stronger than 1:5,000,000

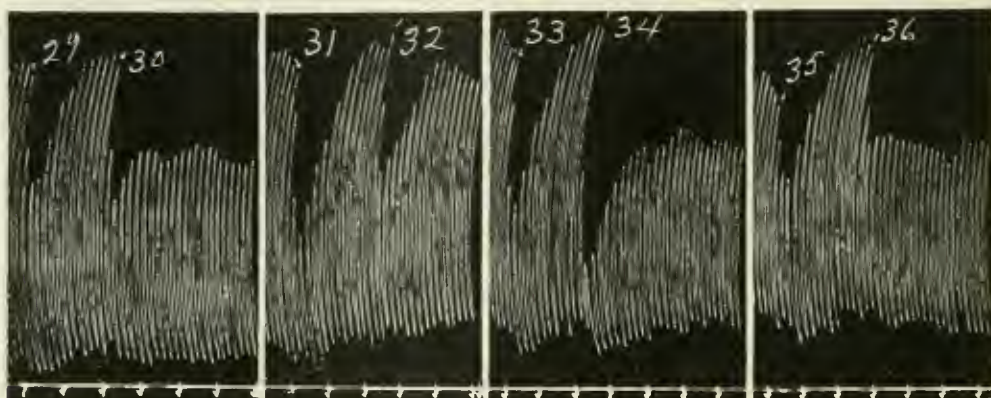


FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT 471

At 29, 31, 33 and 35 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 30 and 36 by the 4th adrenal blood specimen (collected $4\frac{1}{2}$ minutes after injection of physostigmine); at 32 by indifferent blood to which was added adrenalin to make a concentration of 1:6,250,000; at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:3,750,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

(observation not reproduced) and somewhat weaker than 1:3,750,000 adrenalin (fig. 8). It was taken at 1:4,000,000, corresponding to an output of 0.0012 mgm. per minute for the cat, or 0.00044 mgm. per kilogram per minute, more than 4 times the initial output. The sixth specimen, obtained 34 minutes after administration of physostigmine, was much stronger than the fourth, even when diluted with 3 volumes of the indifferent blood, to compensate for the diminished rate of blood flow through the adrenals during its collection (fig. 9). It was found to be decid-

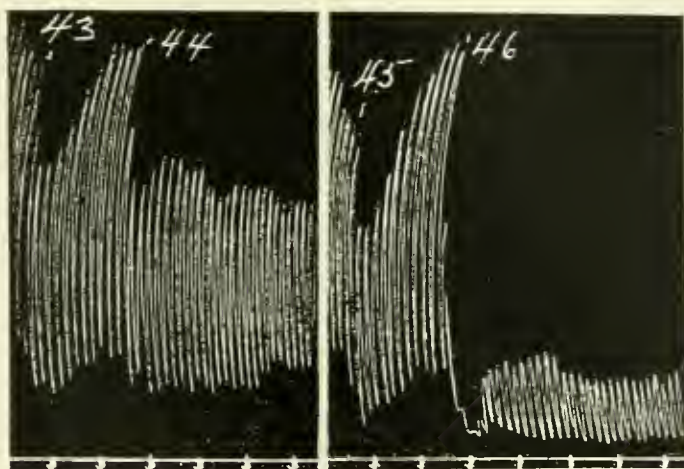


FIG. 9. INTESTINE TRACINGS. BLOODS FROM CAT 471

At 43 and 45 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 44 by the 4th adrenal blood specimen (collected 4½ minutes after injection of physostigmine) at 46 by the 6th adrenal blood specimen (collected 34 minutes after injection of physostigmine) diluted with 3 volumes indifferent blood. All the bloods were diluted with 3 volumes Ringer (the 6th specimen after diluting with indifferent blood). Reduced to two-thirds.

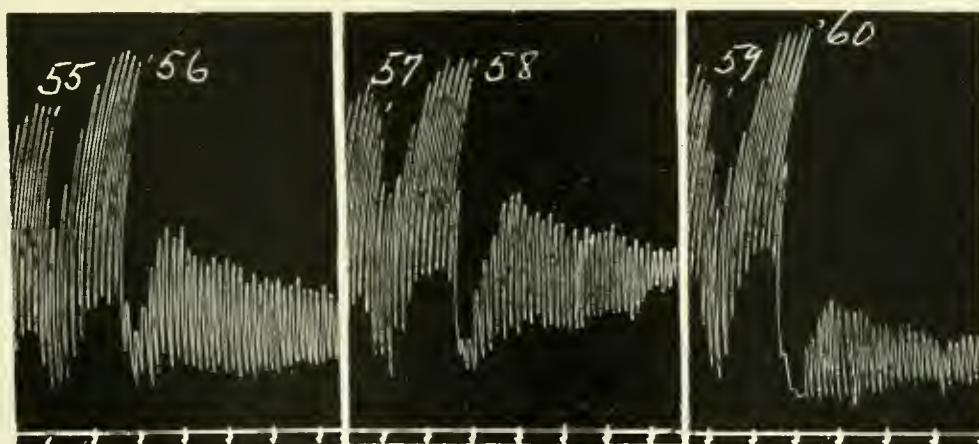


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 471

At 55, 57, and 59 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 56 by the 6th adrenal blood specimen (collected 34 minutes after injection of physostigmine) diluted with 7 volumes indifferent blood; at 58 by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000; at 60 by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenal blood after diluting with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

edly stronger than 1:625,000, stronger than 1:310,000 (fig. 10, confirmed by several pairs of observations not reproduced), weaker than 1:156,000, not unlike 1:235,000. It was taken at 1:250,000, representing an output of 0.004 mgm. per minute for the cat, or more than 0.0014 mgm. per kilogram per minute, that is 14 to 15 times the initial output.

Other experiments with subcutaneous injection of the drug are referred to in the next section.

THE POINT OF ACTION OF PHYSOSTIGMINE ON THE EPINEPHRIN-SECRETORY MECHANISM

It has been stated by Tscheboksaroff (3) that physostigmine increases the output of epinephrin in dogs after section of the splanchnic nerve, "apparently by exerting an exciting action immediately upon the chromaffin cells of the medulla." The action of physostigmine is only discussed incidentally and very briefly by him in his otherwise excellent paper upon "the secretory nerves of the adrenals." The few numerical data given (in one instance the sense seems to be inverted by a verbal error) are quite insufficient to demonstrate the peripheral action of the drug upon the adrenals, even if we could assume that in the dog section of the major splanchnic completely severs the epinephrin-secretory fibers of the corresponding adrenal, which we certainly cannot assume in the cat. The fact that physostigmine exerts a peripheral action on some other structures cannot, of course, be transferred without proof to the adrenal.

We adopted two procedures to test the question: *a*, producing an effect on the output by physostigmine and then denervating the glands, in order to see whether the physostigmine effect persisted; *b*, denervating the glands and then observing whether physostigmine was still capable of increasing the output of epinephrin. By neither method were we able to demonstrate any definite effect of physostigmine when the adrenal nerve supply had been severed as completely as possible.

Condensed protocol. Cat 479; male; weight, 2.55 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein. Cut both vago-sympathetics. Obtained indifferent blood.

- 9.30 a.m. Cava pocket completed. Collected adrenal blood.
- 9.31½ a.m. First specimen, 1.4 gram in 30 seconds (2.8 grams per minute).
- 9.32 a.m. Second specimen, 4.75 grams in 2 minutes (2.37 grams per minute). Blood pressure 102 mm. of mercury.
- 9.37 a.m. End of subcutaneous injection of 4 mgm. physostigmine sulphate. Blood pressure 102 mm. of mercury.
- 9.40 a.m. Blood pressure 112 mm. of mercury.
- 9.45 a.m. Blood pressure fell rapidly to 71 mm. Tied coeliac axis and superior mesenteric artery. Blood pressure rose to 100 mm. Heart rate much slower. Salivation, lachrymation and muscular twitching present. Pupils wide and mictitating membranes forward.
- 10.00 a.m. Heart rate very slow. Profuse salivation and lachrymation; pupils less wide and mictitating membranes retracted.
- 10.00½ a.m. Third specimen, 0.65 gram in 30 seconds (1.3 gram per minute).
- 10.01 a.m. Fourth specimen, 3.15 grams in 3 minutes. (1.05 gram per minute). Blood pressure 64 mm. of mercury.
- 10.11 a.m. Sectioned nerves to both adrenals.
- 10.12 a.m. Pupils smaller, mictitating membranes retracted; other symptoms quite marked. Pads sweating.
- 10.15½ a.m. Fifth specimen (not weighed).
- 10.16 a.m. Sixth specimen, 2.7 grams in 4 minutes. (0.67 gram per minute.) Blood pressure 43 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.402 gram.

In cat 479 the second adrenal specimen was stronger than 1:6,250,000, decidedly weaker than 1:3,750,000, and was assayed at 1:5,000,000 adrenalin, corresponding to an output of 0.00047 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute. Physostigmine (1.6 mgm. per kilogram) was now injected subcutaneously, and 24 minutes thereafter the fourth

specimen was collected. It was found to be decidedly stronger than 1:710,000 adrenalin, stronger than 1:375,000 and probably than 1:355,000, weaker than 1:175,000, decidedly stronger than 1:500,000 (fig. 11). The concentration of epinephrin in this specimen was so great that it was found necessary to dilute it first with six and ultimately with nine volumes of indifferent blood, in order to make a good assay. Thus, in figure 11, observations 40 and 46, the real difference between the concentration of the blood and 1:175,000 adrenalin is not strikingly brought out because the dilution (with 6 volumes of indifferent blood) is insufficient. The fourth specimen was finally assayed at 1:325,000, corresponding to an output of 0.0032 mgm. per minute for the cat, or more than 0.0012 mgm. per kilogram per minute, about 6 times the initial output.

Immediately after the collection of the fourth adrenal blood specimen, the nerves to both adrenals were divided and adrenal vein blood then collected. All the nerves seen coming to the semilunar ganglia, including the splanchnics (major and minor) were severed.

The sixth specimen, collected after division of the nerves, had a much smaller concentration of epinephrin than the fourth specimen, in spite of the fact that the blood flow during the collection of the fourth specimen was the greater. Even when the fourth specimen was diluted with six volumes of indifferent blood, it was much stronger than the sixth specimen diluted with three volumes of indifferent blood (fig. 11, observations 32 and 34). The sixth specimen was shown to be weaker than 1:3,125,000 and stronger than 1:6,250,000 adrenalin (fig. 12). It was taken at 1:5,000,000, corresponding to an output of 0.00013 mgm. per minute for the cat, or 0.00005 mgm. per kilogram per minute, only one-twenty-fourth of the output before denervation, at the time of collection of the fourth specimen, and only about a quarter of the output at the beginning of the experiment before physostigmine was given.

In another experiment (cat 476) performed in the same way, the subcutaneous injection of 0.5 mgm. of physostigmine sulphate into a cat weighing 2.15 kgm. caused a distinct increase in the epi-

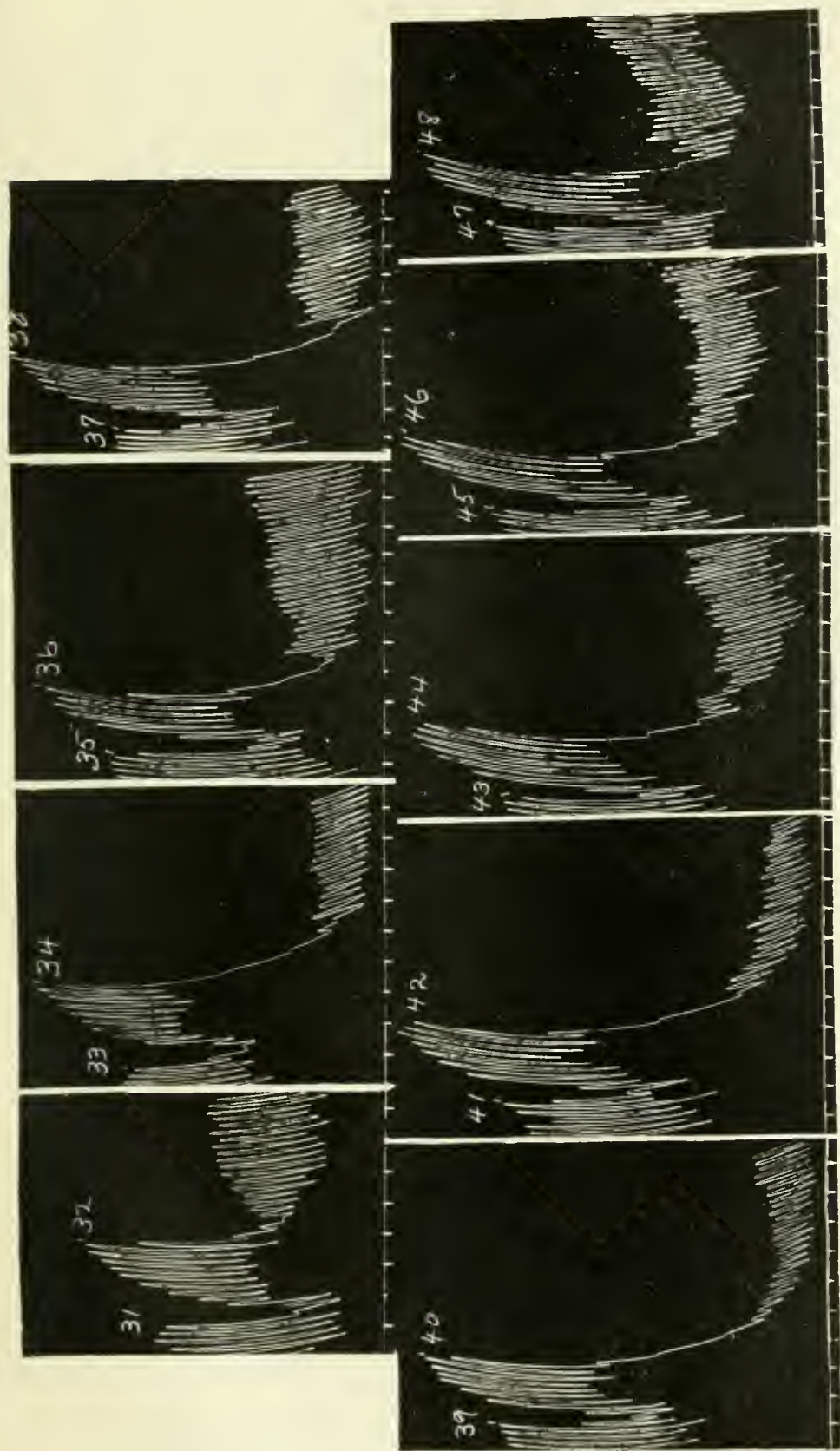


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 479. At 31, 33, 35, 37, 39, 41, 43, 45 and 47, Ringer was replaced by indifferent blood (obtained after administration of physostigmine) and this at 32 by the 6th adrenal blood specimen (collected after section of nerves to adrenals) diluted with 3 volumes indifferent blood; at 34 and 40 by the 4th adrenal blood specimen (collected 24 minutes after injection of physostigmine) diluted with 6 volumes indifferent blood; at 36 and 48 by indifferent blood to which was added adrenalin to make a concentration of 1:5,000,000; at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000; at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000; at 44 by the 4th specimen diluted with 9 volumes indifferent blood; at 46 by indifferent blood to which was added adrenalin to make a concentration of 1:3,750,000. All the bloods were diluted with 3 volumes Ringer (the adrenal bloods after diluting with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

nephren output. Both adrenals were now denervated, the semilunar ganglia being excised, and specimens of adrenal blood collected. This blood (despite the slow blood flow, less than half that before denervation) gave no inhibition whatever of the intestine segment, although the specimen taken just before the denervation gave a good reaction and had a concentration estimated at about 1:1,000,000 adrenalin.

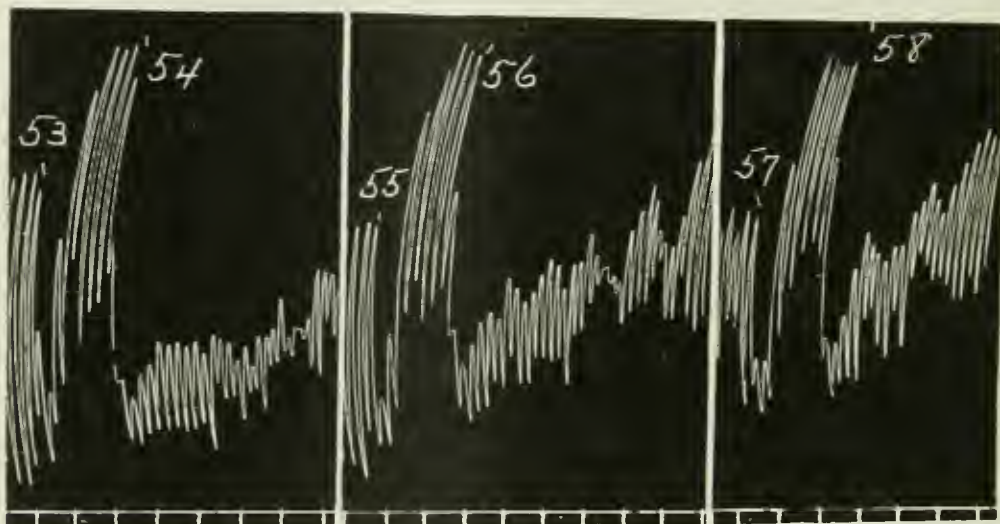


FIG. 12. INTESTINE TRACINGS. BLOODS FROM CAT 479

At 53, 55 and 57 Ringer was replaced by indifferent blood (obtained after injection of physostigmine) and this at 54 by indifferent blood to which was added adrenalin to make a concentration of 1:12,500,000; at 56 by the 6th adrenal blood specimen (collected after section of nerves to adrenals) diluted with 3 volumes indifferent blood; at 58 by indifferent blood to which was added adrenalin to make a concentration of 1:25,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenal blood after diluting with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

Condensed protocol. Cat 486; male; weight 4.21 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein. Cut both vago-sympathetics. Obtained indifferent (jugular) blood.

9.55 a.m. Cava pocket completed. Collected adrenal blood.

9.56½ a.m. First specimen, 5.93 grams in 20 seconds (17.8 grams per minute).

9.57 a.m. Second specimen, 9.65 grams in 45 seconds (12.9 grams per minute). Blood pressure 138 mm. of mercury.

10.07 a.m. Sectioned nerves to both adrenals.

- 10.12 $\frac{1}{2}$ a.m. Third specimen, 1.8 gram in 30 seconds (3.6 grams per minute).
- 10.13 a.m. Fourth specimen, 6.1 grams in 2 minutes (3.05 grams per minute). Blood pressure 90 mm. of mercury.
- 10.20 a.m. End of intravenous injection of 2.25 mgm. physostigmine sulphate. Blood pressure 126 mm. of mercury.
- 10.23 $\frac{1}{2}$ a.m. Fifth specimen, 2.15 grams in 30 seconds (4.3 grams per minute).
- 10.24 a.m. Sixth specimen, 5.43 grams in 90 seconds (3.62 grams per minute). Blood pressure 71 mm. of mercury. Profuse salivation and lachrymation and marked muscular twitching present. Marked increase in intestinal peristalsis.
- 10.38 $\frac{1}{2}$ a.m. Seventh specimen, 1.25 gram in 30 seconds (2.5 grams per minute).
- 10.39 a.m. Eighth specimen, 4.7 grams in 2 minutes (2.35 grams per minute). Blood pressure 54 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.714 gram.

In cat 486 the glands were denervated before the administration of physostigmine. The second adrenal blood specimen, collected before denervation, was shown to be decidedly weaker than 1:13,300,000, weaker than 1:20,000,000 adrenalin (the blood flow was exceptionally great) and not much different from 1:40,000,000, corresponding to an output of 0.00032 mgm. per minute for the cat, or 0.000075 mgm. per kilogram per minute. This is not much more than one-third of the average normal output in etherized cats, and therefore a good opportunity was provided for a large percentile increase under the influence of physostigmine, administered after denervation, if this drug is really capable of increasing the output of epinephrin after section of the nerves. This did not occur, however. The fourth specimen, obtained after section of the nerves, but before the injection of physostigmine, was found to be weaker than 1:53,000,000 and even somewhat weaker than 1:100,000,000 (confirmed by several sets of observations). Even taking it at 1:100,000,000, the output would only be 0.00003 mgm. per minute for the cat, or 0.000007 mgm. per kilogram per minute, less than one-tenth of the output before denervation.

The sixth specimen, collected 4 minutes after giving physostigmine, did not differ much in concentration from the fourth. Since the blood flow during its collection was also about the same as for the fourth specimen the physostigmine at this stage had produced practically no change in the output (it was calculated at 0.000008 mgm. per kilogram per minute). The eighth specimen, collected about 20 minutes after administration of the drug, was shown to be stronger than the sixth specimen (the blood flow was less), decidedly weaker than 1:13,300,000, stronger than 1:53,000,000. It was approximately assayed at 1:30,000,000, corresponding to an output of 0.00007 mgm. per minute for the cat, or 0.000016 mgm. per kilogram per minute, only about one-fifth of the low initial output. The fact that the output for the eighth specimen is double that for the fourth is no evidence that physostigmine can exert a distinct stimulating action on the adrenal medulla in the absence of innervation. For the denervation not being quite complete, as indicated by the persistence of a small epinephrin output after division of the nerves, the central action of physostigmine would, of course, make itself felt over any portion of the nervous path which had escaped section.

SUMMARY

After the administration of physostigmine, either intravenously or subcutaneously, in cats, the epinephrin output of the adrenals was found to be augmented (to as much as 10 to 15 times the initial output). The stage of augmentation is prolonged and is preceded by a transient diminution.

No evidence was obtained that after section of the splanchnics and other nerves going to the adrenals, physostigmine can increase the epinephrin output by peripheral action.

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OBSERVATIONS ON THE EFFECT OF IPECAC IN THE TREATMENT OF INFECTIOUS ENTERO-HEPATITIS (BLACKHEAD) IN TURKEYS

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The prevalence of the disease among turkeys commonly known as blackhead can be traced back definitely only a few decades. While it can hardly be doubted that the disease existed among fowl before that time: nevertheless its inroads on commercial stock were not noticed until a short time prior to 1892. During this year an epidemic of the disease had reached such proportions in Rhode Island that the turkey raising industry was definitely hampered. So serious had the situation become that the attention of the Poultry Division of the Rhode Island Experiment Station was attracted to it, and issued a bulletin concerning it. In this bulletin and a subsequent one appearing the following year, Cushman (1), recognized and described quite clearly the chief clinical and pathological findings. Concerning the clinical aspects of the disease he remarks that "when once started it is apt to be quickly fatal. There is a diarrhea, weakness, no appetite, and the face and comb change from a bright scarlet to a dark purple. As soon as this dark purple is shown the bird soon dies unless vigorous remedies are promptly given." That Cushman recognized the intestinal manifestations of the disorder is evidenced by his references to it, both as a "bowel trouble" and as cholera. His autopsies of birds dying of the disease revealed the localization of the inflammation in the caeca and the spots of infection in the liver. As a result of the investigations carried on at the Rhode Island Station, Dr. Theobald Smith (2), then Director of the Bureau of Animal Industry became interested in the problem and devoted a part of his time during 1894 to the study of it. In

his bulletin on the subject which appeared the following year, Smith gives us an excellent description not only of the symptoms manifested by the birds during infection, but also of the pathological lesions found in the intestines and liver, from which he classified the malady as an entero-hepatitis. During the course of his researches, this investigator discovered and described an organism, named by him "ameba meleagridis" which appeared so constantly and was so closely associated with the lesions characterizing the disease that he concluded that it was the causative agent. Some years later, 1910, Cole and Hadley (3) disputed the deductions made by Smith and denied the presence of ameba in the lesions of blackhead. These writers on the contrary had noted the presence of coccidia in cases of the disease throughout the intestines, liver and occasionally the oviduct. The infective cycle of this organism was very carefully studied by these authors and from their observations they conclude that "the ameba meleagridis (described by Smith) was in reality, the schizont stage of a coccidium." Smith answered their criticism of his work five years later (1915) and unquestionably demonstrated that the coccidium is not the true cause of blackhead and when found during the course of the infection is present only as a secondary invader. In this last publication, the writer gives the results of further control experiments which strengthen his original conception concerning the causation of the disease namely through infection of the alimentary canal with the ameba meleagridis, and he leaves little doubt in the minds of students of the subject of the correctness of his deductions.

Two important communications on the experimental transmissions of the infection were published by Moore (4) (1896) and Chester and Robin (5) (1901). The former writer was able to transmit the disorder from turkey to turkey by feeding the diseased liver and caeca, while the latter by a similar method were able to transmit the disease from turkeys to other fowl. Both of these papers are extremely important from an experimental standpoint and will be referred to again later in this communication.

It is noteworthy that during the entire thirty-year period of investigation of blackhead, the headway gained in combating it has been practically nil. The clinical, pathological and bacteriological investigations of the malady have yielded very gratifying results but the mortality rate continues practically as high now as it did three decades ago when the inroads of the disease began to show their devastating effects among the flocks of the Rhode Island farmers. Numerous therapeutic measures have been suggested but the trial of none of them has resulted in definitely checking the course of the infection, although Cole and Hadley report that in several cases birds suffering from blackhead fully recovered after the use of capsules containing sulphur and the sulphates of iron and quinine and others containing salicylate of soda in combination with either the sulphates of iron or benzonaphthol. Salol was also used by these writers. Curtice (6) states that treatment has not given satisfactory results and recommends a dose of either epsom salts or castor oil before giving any of the drugs recommended above. He also advises the addition of catechu to the drinking water. Kaupp (7) recommends a combination of sodium, calcium and zinc sulpho carbolate, from which he has received good reports. All of these, as well as other authors, however, have practically ceased to look for results from medication and have concentrated their efforts toward prevention, their chief attention being directed to (1) feeding, (2) prevention of early infection by cleansing the eggs well before incubating, which is done artificially, followed by the use of hens for brooding, and (3) prevention of late infection by keeping the poults off of infected ground, using instead brood-houses equipped with wood flooring which can be disinfected from time to time.

The writers' experience with blackhead began about fourteen years ago when it was first observed by them among some turkeys which were being raised in Baltimore County, Maryland. The disease was quite prevalent among the flocks throughout that part of the state and caused undoubtedly the major portion of the losses among turkeys. The farmers referred to it by no particular name, the malady being attributed to a predisposition

on the part of the turkey to catch cold and die if it got its feathers or feet wet while walking in the dew laden grass or if caught in a rainstorm. Autopsies on the birds dying in our flocks at that time demonstrated clearly enough that the disease which was carrying off our poults was quite definite in its pathological manifestations, for in nearly every bird examined the striking inflammatory changes in the caeca and liver, which were later learned by us to be characteristic of blackhead, were present. Time did not permit us then to investigate further this interesting disease and the general conception held by the farmers of the vicinity namely that the disease was caused by exposure of the turkeys to dampness was shared by us, for it was indeed striking how the mortality would increase among a brood which had been caught in a rainstorm or had been allowed to wander off in the morning before the grass had thoroughly dried. While no exact data are available now it can safely be said that the toll exacted by the disease at that time among the young poults was not less than 50 per cent.

Several years after the experience in Maryland one of us started to raise turkeys in San Diego County, California. It was not long before it became evident that the young turkeys were being carried off by a disease in all respects similar to that which had prevailed among the flocks in Maryland. Since the climatic conditions in this particular part of California are so far as turkey raising is concerned, ideal, the old theory entertained in the East for the causation of the disease was no longer tenable, and an earnest investigation into the nature of the malady was instituted, the results of which are the basis of this present communication.

The epidemiological features demonstrated on our ranch in California have been on a small scale, those of most of the neighborhoods into which turkey culture has been introduced. Beginning with a small apparently non-infected flock on clean unused soil the culture of the birds during the first year was uneventful. In a short time, however, the disease made its appearance and became established among the flock and in the soil of the ranch from which it seemed almost impossible to ever rid it. This

gradually strengthening of its hold on a flock by the disease is very well shown by the following brief history of our own ranch.

In the spring of 1914 four pullets and a gobbler were brought from Ohio and placed on a ranch on which to our knowledge, no turkeys had ever been grown before. From these were raised forty birds, a number of young ones being lost from vermin. None of these birds died of blackhead nor were there any evidences, clinically, of it among the flock this year. During the following year (1915), although no new birds or eggs had been brought to the ranch, blackhead appeared and more than half of the young flock and one of the old birds were lost. Autopsies were made on all of the birds, and in all the lesions of either the caeca or the liver characteristic of blackhead were found.

In the spring of 1916 more than eighty birds were hatched, of which more than sixty reached the age at which they were able to roost in trees before the disease made its appearance. The late rains coming at the same time helped undoubtedly to lower the vitality of the flock so that only one bird was saved out of the entire spring's hatch. All of the old birds were disposed of and the ranch was moved to a ten-acre lot, about one-quarter of a mile away, on which certainly no turkeys had been raised for twenty years, and probably none even before that time. A small beginning was again made with turkeys brought from the East (New York State), the birds being bought from two different breeders. This year thirty-seven birds were raised and although some were lost from exposure and other causes, autopsies showed no blackhead. The next year some new birds from the East were added to the flock. All the birds, both our own from the last year's flock and the recent acquisitions were well matured and at no time showed symptoms of blackhead. Excluding newly born turkeys which die almost immediately after leaving the shell, two hundred and ten birds were hatched this year. All of these but seventy-two were lost, one hundred at least dying from blackhead and the remainder from chicken pox and blackhead combined.

The same conditions repeated themselves in 1919. This year two hundred and nine young turkeys were given to hens and

kept in pens for ten days before being allowed to run in the alfalfa. All remained healthy until they were about six weeks old when they were attacked by the disease from which all were lost but fifty-two. It cannot be questioned that the mortality would have been even heavier had we not instituted the measures of which we shall speak later.

For the early diagnosis of the disease it was found that careful inspection of the droppings each day was of great assistance. The first symptom that a bird coming down with blackhead usually shows is a slight lameness of the leg, usually the left. Just about this time the droppings become liquid, frothy and frequent so that it has frequently happened in our experience that detection of such droppings in an enclosure has led to the successful search of a sick turkey. The number of times that this early diagnosis has been confirmed by death and subsequent autopsy has not been without its benefit for, by its use, a great many birds subsequently were subjected to treatment at a time when the best results could be expected. The characteristic droppings were the first symptom in about one-half of the cases observed and has been typical of the disease in about 75 per cent of the turkeys infected. Following the lameness the turkey becomes sluggish and the head frequently, but not always, becomes discolored, the fleshy parts taking on a bluish-green cast. During the course of the next day or two, the feathers on the rump begin to rise and later the wings and tail droop; the head is carried less erect and the eyes remain closed most of the time, opening, when they do, slowly and languidly. Death sometimes occurs within twenty-four hours after the onset, but most commonly the turkey succumbs on the second or third day. Turkeys sacrificed at the different clinical stages outlined above have shown a characteristic progression of the infection. If sacrificed early when the bird begins to limp, the caeca alone were noted to be inflamed. Later when the feathers begin to rise over the rumps, involvement of the liver can be found and by the time the wings and tail droop, the liver is usually filled with necrotic areas.

During the epidemics mentioned above various methods were employed, unsuccessfully, for combating the diseases. The isolation of all sick birds was a routine; all dead birds after being autopsied were destroyed. As it was believed extensively that food caused the disease, experiments were instituted to determine to what extent it was true as regards our own flock. On three different occasions soft food, such as egg mash, was given for a period of a month at a time at five-months intervals. The experiments resulted in an increase of the disease, birds of six and eight months of age frequently succumbing after such a diet. Various tonics recommended both as a cure and prevention were resorted to without any noticeable results. Laxatives, sour milk, buttermilk and sweet milk were also used without any apparent benefit. It was toward the end of the season of 1919, when it seemed the entire flock was destined to be exterminated that attention was drawn to Dr. Theobald Smith's, researches on the etiology of the disease. His discovery of the ameba in the lesions of blackhead suggested the use of ipecac which has proved so valuable a remedy in cases of human enteritis of amebic origin. Accordingly it was determined to treat all birds showing signs of the disease with ipecac. Most of the damage had already been done, it is true, as our experiments did not begin until September, 1919, at which time aside from the old flock, which was now being attacked also, there remained only fifty-two young birds out of two hundred and nine hatched in the spring. As the epidemic showed no signs of abating, however, there was still enough clinical material to work on. A routine was established on the ranch whereby any bird showing symptoms of blackhead was immediately separated from the rest of the flock and given by mouth ten drops of the fluid extract of ipecac three times a day. This dosage was continued for three days after which it was decreased to ten drops twice a day for three days and later once a day for the same period.

We were indeed surprised when, after treating six or eight turkeys with ipecac we found that instead of needing autopsies, the birds were recovering and could later be turned back with the flock. Of the fifty-two young birds forming our supply of

clinical material, thirty-two were attacked by the disease. Under the ipecac treatment, twenty-nine of these recovered completely, making a mortality of less than 9 per cent. Of five of the old birds contracting the disease during the same period three were cured while two were lost; those lost were very severe cases almost fulminating in character and succumbed in less than two days. Twenty of the young birds never contracted the disease.

In order to control the results we were getting, an experiment was arranged with the idea of determining, if possible, if the disease could be prevented. Recalling that Moore as well as Chester and Robin were able to transmit the disease from fowl to fowl it was decided to infect a group of birds by the method used by these writers, after which ipecac medication could be instituted and experimentally controlled. For this investigation we obtained eighteen apparently healthy, nearly full grown turkeys from a ranch several miles from our own. To each of these was fed an equal quantity of the ground caeca and liver of a turkey dying the same day of blackhead. The birds were then divided into three equal groups and placed in three pens formerly used for quarantining turkeys with the disease. To each of the turkeys in the first pen were given ten drops of the fluid extract of ipecac by mouth once a day for three days. The turkeys in the second pen were fed with a mash containing three teaspoonsful of the powdered ipecac to the quart for three successive days. This food was kept constantly before them and they ate of it quite as eagerly as they would other food. The turkeys in the third pen were used as controls and were given the ordinary food. From the first pen we lost one bird on the fifth day which did not die from blackhead but from some other disease which could not be determined from autopsy. The rest of the birds in this pen remained healthy. All of the birds in the second pen remained healthy. In the third pen four of the birds died of blackhead within a period of less than sixty days. The two remaining turkeys in this pen remained healthy. At the expiration of two months' observation all of the birds in the three pens were banded and turned loose. There were no more

losses among them. The mortality percentage ($66\frac{2}{3}$ per cent) of experimental blackhead among the control turkeys in the third pen is somewhat higher than that (50 per cent) of Moore's first experiment in which one of the two turkeys fed diseased viscera succumbed, and somewhat less than that of his second experiment in which a mortality of 75 per cent was obtained by feeding excrement from infected turkeys. It is interesting also to note that the four turkeys in our group died as the result of the experimental inoculation within sixty days, a period only slightly longer than that (six weeks) noted by Moore in his investigation. The hen infected by Chester and Robin died in twenty-eight days but on account of the difference in species a comparison of time elements in this instance is hardly safe.

The results obtained so far in being able not only to cure but also to apparently prevent the infection by the administration of ipecac while not conclusive were nevertheless encouraging. As the season was about over, however, and as there were no young birds available for experimentation, the investigation was not resumed until the following spring (1920). Administering and caring for each individual bird stricken with the disease during even a moderate epidemic is no small task and to be done correctly needs personal supervision. As neither of us could be at the ranch daily it was decided to hatch only a few turkeys and, using the last experiments given above as a basis, to try preventive measures rather than permitting the disease to get a start. Accordingly, the same routine was established as that used in the second pen described above, namely, the administration of powdered ipecac with the food. The dosage was placed arbitrarily at a teaspoonful of the powdered drug for each unit of twenty turkeys twice a week, no difference being made for the age or size of the birds. The poults were hatched artificially as formerly after which they were given to hens and allowed to run over the same ground used by the infected birds of the years previous. Early in May, sixteen birds were hatched and after ten days confinement they were placed with hens and allowed to run in the usual enclosure. The latter part of the month twenty-two more were hatched and treated in the same

way. They were all watched very closely and kept clean of vermin. These birds were all raised and none of the flock have shown any signs of blackhead. In July sixty-eight more birds were hatched and turned loose in alfalfa with turkey hens. Members of this group began disappearing as rapidly as two and three a day. The carcasses were never found and it was not until a neighbor's cat was discovered invading the flock that the cause of the losses was explained. At this time six birds were left all of which were raised.

Up until the time of the present writing, February, 1921, blackhead has not made its appearance in a single individual of either the old or young turkeys on the ranch, since the institution of prophylactic feedings of ipecac. As these turkeys are all raised for show and breeding purposes none of them were marketed, so that the observation of the turkeys hatched last May extends over a period of nine months. This absence of the disease has not been experienced, however, by other ranches in this vicinity, for they have been having the same difficulty as formerly, the losses from "spotted liver disease," as the malady is designated locally, being everywhere high.

To sum up, it has been demonstrated that infectious enterohepatitis of turkeys can be cured by the administration of suitable doses of ipecac. It has also been shown that in turkeys experimentally infected by the feeding of viscera taken from a turkey succumbing of the disease can be prevented from contracting the disease by either the oral administration of the fluid extract of ipecac or by the ingestion of the powdered drug with the food. Further it can be said that the disease failed to make its appearance among the turkeys on a ranch which for several years previous had been visited by epidemics of the disease in a virulent form, the prevention being attributed to the prophylactic administration of ipecac with the food.

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THE COMPARATIVE TOXICITY OF THYMOL AND CARVACROL (ISOTHYMOL)

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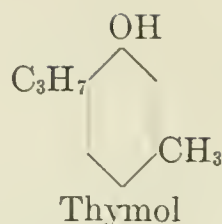
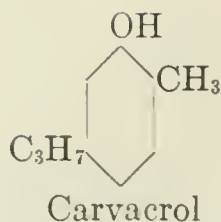
INTRODUCTION

It is evident from the literature that new or additional specifics for the treatment of hookworm disease are desirable. Thymol, which for many years has been the principal remedy, became almost unobtainable during the war when our imports from Germany were cut off. This condition led to an extensive substitution of oil of chenopodium. Either of these drugs may produce serious toxic effects, and either, especially thymol, may fail to expel the worms as desired. We are dependent upon our imports for thymol which in time of war may fail to reach us, while oil of chenopodium is a variable product, sometimes being more toxic and sometimes less efficient. The cost of thymol at times is high enough to become an important factor, and serious after-effects such as blindness have been reported to follow the use of oil of chenopodium.

At a time when these conditions prevail, Hixson and McKee (1918) report a process by which carvacrol may be produced from spruce turpentine, and mention the possibility of its use as a substitute for thymol. No data being found in the literature in regard to its toxicity, a comparative study of carvacrol with thymol was undertaken, at the suggestion of Dr. McKee who has kindly supplied us with the carvacrol used.

PROPERTIES

Carvacrol, as may be seen by the following formulae is an isomer of thymol:



As it solidifies at 1.0°C . and boils at 236°C . it is encountered as a liquid whereas thymol having a melting point of 49.6°C . and a boiling point of 231.8°C . is a solid even at body temperature. Carvacrol has a pungent aromatic taste, which much resembles that of thymol and possesses a distinct local anesthetic property as may be noticed when the drug is applied to the tongue. At the time of distillation it is a colorless oil which upon long standing assumes a reddish brown color. It has a peculiar odor not unlike that of thymol but much less pleasant. Thymol according to Seidell (1919) is soluble to the extent of about 880 parts per million in water at 20°C . No definite statement in regard to the solubility of carvacrol in water has been found, but with the highest concentrations used in these experiments, which was 500 parts per million, a perfectly clear solution was obtained. The U. S. Dispensatory (1918) quotes Martindale as stating that carvacrol is almost as actively germicidal as its isomer thymol, but the data upon which this statement is based have not thus far been found by the writer. Hixson (1918) includes the statement that "Recent comparative tests have shown carvacrol to be practically equal to and, in some cases, to possess greater antiseptic values than thymol," but this conclusion is based on only four viability tests on bacteria. Sollman (1919), whose results were published after the present study was under way, concludes that carvacrol ranks with oil of chenopodium and thymol in toxicity for earthworms, that probably it is more irritant and toxic for dogs than thymol, and that it deserves a careful clinical trial as an anthelmintic substitute for thymol. The writer has been advised that clinicians have found that patients objected to its use on account of the taste. It is possible that a method of administration might be adopted which would obviate this objection. The factors, then, which indicate that carvacrol may be used as a substitute for thymol

are as follows: Its source of supply in this country is assured; the raw materials from which it may be made are inexpensive; it is a liquid instead of a solid as thymol, at body temperature, which gives it a better chance of coming in contact with all parts of the intestinal wall; it has a distinct local anesthetic property which combined with its anthelmintic action indicates a high efficiency; and, being an isomer of thymol its toxicity, as well as anthelmintic properties as shown by Sollman, is probably quite similar to that of thymol. In connection with the present toxicity experiments on rabbits and paramecia, it seemed that additional evidence in regard to its anthelmintic action as indicated by earthworms might be valuable.

EXPERIMENTS ON RABBITS

Dogs were found not to be suitable for this work since they vomited soon after the drug was given which of course made it possible that some was lost. For this reason rabbits were chosen for further work. Both drugs have been introduced into the stomach by three methods. Sometimes they were given in a 50 per cent solution in olive oil in gelatin capsules, sometimes in full strength in gelatin capsules, and at other times by means of a small inelastic catheter attached to an accurately graduated syringe. In case of thymol the catheter method could be used only when the drug was dissolved in oil while this method was applicable to carvacrol either in full strength or in oil. No comparison of results has been made to determine whether or not any difference in toxicity has resulted from these different methods of administration. The introduction by catheter and syringe, both being completely filled, has proven the most satisfactory from the standpoints of accuracy and convenience.

In all 106 rabbits have been used, grouped according to the drug employed into 4 series: those receiving 50 per cent thymol dissolved in olive oil, those receiving thymol in the form of powder, those receiving 50 per cent carvacrol in olive oil, and those receiving carvacrol in pure form. The dose for each has

ranged from 0.25 gram to 3 grams per kilo. A considerable individual variation in susceptibility is noticed which is probably due in part at least to the variable amount of food in the stomach. The length of time survived for each animal, or the time the animal was kept under observation, varied from a few hours to several weeks.

It may be observed from table 1 that in doses of 0.25 gram per kilo 5 received thymol and 5 carvacrol. Only 1 rabbit died during the period of observation which extended over sixty-five days. This rabbit lived fifteen days after receiving thymol which probably means that neither the thymol nor carvacrol in this dose was responsible for any deaths.

The next higher dose was 0.5 gram per kilo and included 30 rabbits. Among the 14 carvacrol animals receiving this dose 2 died within three days while the remaining 12 lived eight days or longer (most of them five weeks) after receiving the drug. Of the 16 thymol rabbits receiving 0.5 gram per kilo none died in less than sixteen days.

Among 11 rabbits which received 0.75 gram per kilo, 5 received the carvacrol and 6 the thymol. The table shows that of the 5 receiving carvacrol, 1 died the second and 1 the fourth day, whereas the other 3 lived twenty-seven days or longer. Of the 6 receiving thymol the first to die lived nine days and the next fifteen days. The other 4 lived twenty days or longer.

Of the animals receiving 1 gram per kilo 8 received carvacrol and 6 thymol. Six of the 8 carvacrol rabbits died within three days and 3 of the 6 thymol rabbits died within six days. All others lived beyond the time when the drug might probably have been the cause of death.

The dose of 1.5 grams per kilo was used in case of carvacrol on 7 and in case of thymol on 5 rabbits. Of the 7 receiving carvacrol 5 were dead within five days and of the 5 thymol animals 2 were dead in seven days. The only other rabbit which may possibly have died from either carvacrol or thymol lived sixteen days after receiving carvacrol.

A still larger dose of 2 grams per kilo was given to 20 rabbits, 10 receiving carvacrol and 10 thymol. All 10 carvacrol animals

TABLE 1

THYMOL						CARVACROL					
In olive oil			Not in oil			In olive oil			Not in oil		
Number of animal	Dose per kilo-gram	Survived	Number of animal	Dose per kilo-gram	Survived	Number of animal	Dose per kilo-gram	Survived	Number of animal	Dose per kilo-gram	Survived
	grams	days		grams	days		grams	days		grams	days
123	0.25	65+				107	0.25	65+			
124	0.25	15				108	0.25	65+			
125	0.25	65+				109	0.25	65+			
126	0.25	65+				106	0.25	65+			
127	0.25	65+				105	0.25	65+	59	0.33	25
45	0.5	120+	37	0.5	24	31	0.5	3	41	0.5	8
48	0.5	18	36	0.5	24	33	0.5	3	40	0.5	9
47	0.5	31	34	0.5	19	32	0.5	10	43	0.5	32
44	0.5	34	38	0.5	18	29	0.5	40	39	0.5	35
46	0.5	54	35	0.5	34	112	0.5	65+	86B	0.5	10
131	0.5	65+	88	0.5	16	115	0.5	65+			
128	0.5	65+				114	0.5	65+			
129	0.5	65+				118	0.5	65+			
130	0.5	65+				116	0.5	65+			
132	0.5	65+									
134	0.75	15	89	0.75	9	119	0.75	65+	102	0.75	2
136	0.75	20				122	0.75	65+			
133	0.75	65+				121	0.75	4			
135	0.75	65+				120	0.75	27			
137	0.75	65+									
10	1.0	76				36	1.0	95	103	1.0	3
12	1.0	37				23	1.0	120+	86E	1.0	20
11	1.0	5				28	1.0	3	92	1.0	18
9	1.0	6				24	1.0	2			
8	1.0	6				25	1.0	2			
138	1.0	65+									
14	1.5	120+				20	1.5	16	93	1.5	2
16	1.5	120+				21	1.5	120+	86C	1.5	$\frac{5}{8}$
17	1.5	64				18	1.5	5			
15	1.5	6				22	1.5	2			
13	1.5	7				19	1.5	3			
6	2.0	79	95	2.0	5	2	2.0	2	104	2.0	$\frac{3}{4}$
144	2.0	3	112	2.0	15	142	2.0	1	115	2.0	4
4	2.0	2	106	2.0	19	1	2.0	2	114	2.0	3
143	2.0	21	126	2.0	11	141	2.0	1	118	2.0	1
5	2.0	4	131	2.0	1	3	2.0	$\frac{3}{4}$	149	2.0	3
145	3.0	1	100	3.0	4	147	3.0	1	62	3.2	3
146	3.0	1	101	3.0	8	148	3.0	1	119	3.0	6

The + sign indicates that the rabbit was discarded on the day indicated and probably lived even longer.

died within four days, whereas 4 of the 10 thymol animals were dead in the same time, one in five days, 4 in eleven to twenty-one days, and one certainly survived all effects since it lived seventy-nine days.

Eight animals received 3 grams per kilo. Four of these received carvacrol and 4 received thymol. All died within eight days.

No statement has thus far been made as to the comparative results when either thymol or carvacrol was given with or without olive oil. This, however, is shown in the table already mentioned. There was no intention of dwelling particularly on this phase, but since both methods were used, attention may be called to the results obtained. In regard to thymol in doses of 0.5 gram per kilo, no animal died in less than sixteen days and the length of time survived varied to such an extent that it seems improbable for thymol to have been the cause of death in any case. Of these 16 rabbits (receiving 0.5 gram per kilo) 10 received the thymol in olive oil and 6 received it in powdered form in gelatin capsules. In doses of 2 grams per kilo, however, 3 of the 5 animals receiving thymol in olive oil died within four days while the same dose without oil in 5 cases produced two deaths in five days and one of these animals showed on post mortem examination that death was probably due to pneumonia. Two rabbits received 3 grams of thymol per kilo in olive oil and both died within one day. In contrast to these of the 2 rabbits which received 3 grams of thymol without olive oil, one lived four and the other eight days. When carvacrol is given in doses less than 2 grams per kilo, the table would indicate that if any difference in toxicity is shown, it would seem to be more toxic without than with the olive oil. This, however, is not the case when given in doses of 2 and 3 grams per kilo. Definite conclusions as to whether or not carvacrol or thymol is more toxic when given with olive oil can not be reached without further experiments. There seems to be no reason why carvacrol, being already in liquid form at body temperature, should be more toxic with an oil. On the other hand, since thymol is a solid at body temperature, it might be expected to be more

toxic in the presence of a solvent as an oil. This was reported by Stiles (1902) to be the case in dogs when thymol was followed by castor oil. Schultz (1915) on the other hand says "It was found that oils in which thymol readily dissolves, if used as a solvent, greatly increased the dose necessary to kill." The question which concerned us more in this connection was the relative toxicity of thymol and carvacrol. It is evident from Table I that there is no striking difference in the toxicity of the two substances when introduced into the stomach of rabbits. A close examination, however, of the whole series apparently shows a slightly greater toxicity for carvacrol than for thymol. This difference is certainly not enough to discourage a clinical trial in cases where conditions can be carefully controlled. As a matter of precaution, the dose used at first should of course be much smaller than the relative toxicity on rabbits would indicate.

EXPERIMENTS ON EARTHWORMS

There is no intention of concluding directly that if carvacrol is toxic for earthworms it is likewise toxic for hookworms in the intestinal tract of man. Thymol, however, is generally known to have such an action and if thymol and carvacrol affect earthworms in the same way then we have reason to believe that they may also act the same on hookworms. Two species have been used, namely: *Helodrilus calignosa* (common garden worm) and *Allobophora foetida* (commonly known as the dung worm). In only a few cases, however, were the former observed and hence all conclusions herein mentioned will refer to the latter. The worms were brought into the laboratory in some of the earth in which they were found and kept in a large evaporating dish covered with a piece of plate glass. This prevents their escape and also keeps the earth from becoming too dry.

Earthworms were used by Straub (1902) for the determination of the relative toxicity of various substances. He used glass dishes of a size such as to allow 50 cc. of the solution tested to fill the dish to a depth of about 3 mm. and states that in well water the worms behaved normally for days.

The method used by Sollman (1919) and later by Macht (1919) consists in placing five worms in 100 cc. of the solution to be tested in a conical urine glass. This method was used in the first few of these experiments but the control experiments carried out by immersing the worms in distilled water, tap water, and tap water containing some of the earth in which the worms live, showed that two worms in each glass did not remain in good condition over long periods of time. In these fluids in some cases the worms were found to be dead the next day. This suggested some cause other than the drug itself. Drowning was suspected and petri dishes were substituted for the urine glasses. Fifty cubic centimeters of the control, or of the solution containing the drug to be tested, were placed in a petri dish 15 cm. in diameter and the dish kept closed. By using 50 cc. in these dishes the control fluids caused no effect even though the worms were confined in them for more than a week. The solution is thus too shallow to complicate the result by a possibility of drowning, yet deep enough to insure an exposure of the worms to the drug at all times, and to prevent an appreciable loss of the drug. All experiments have been made at room temperature which ranges in the neighborhood of 21°C. Fresh solutions of the drugs in distilled water were always used. Before making an observation the worms were immersed in water to remove adhering particles of earth. Two were then placed in each dish and closely observed for a few minutes. Further observations were made at intervals of fifteen minutes for several hours, or until death occurred. The findings for thymol and carvacrol agree in every respect with the possible exception of the length of time required to kill. The response varies in intensity but not in character with the concentration of the solution used. In the strongest solution (500 parts per million) the worms at first make a few frantic efforts to escape. These are quickly followed by whipping and writhing movements which rapidly become more and more feeble until all motion ceases which occurs within ten to fifteen minutes. Incidentally it is of interest to note that within a few seconds after the worms are placed in either solution they begin to discharge a round mass of

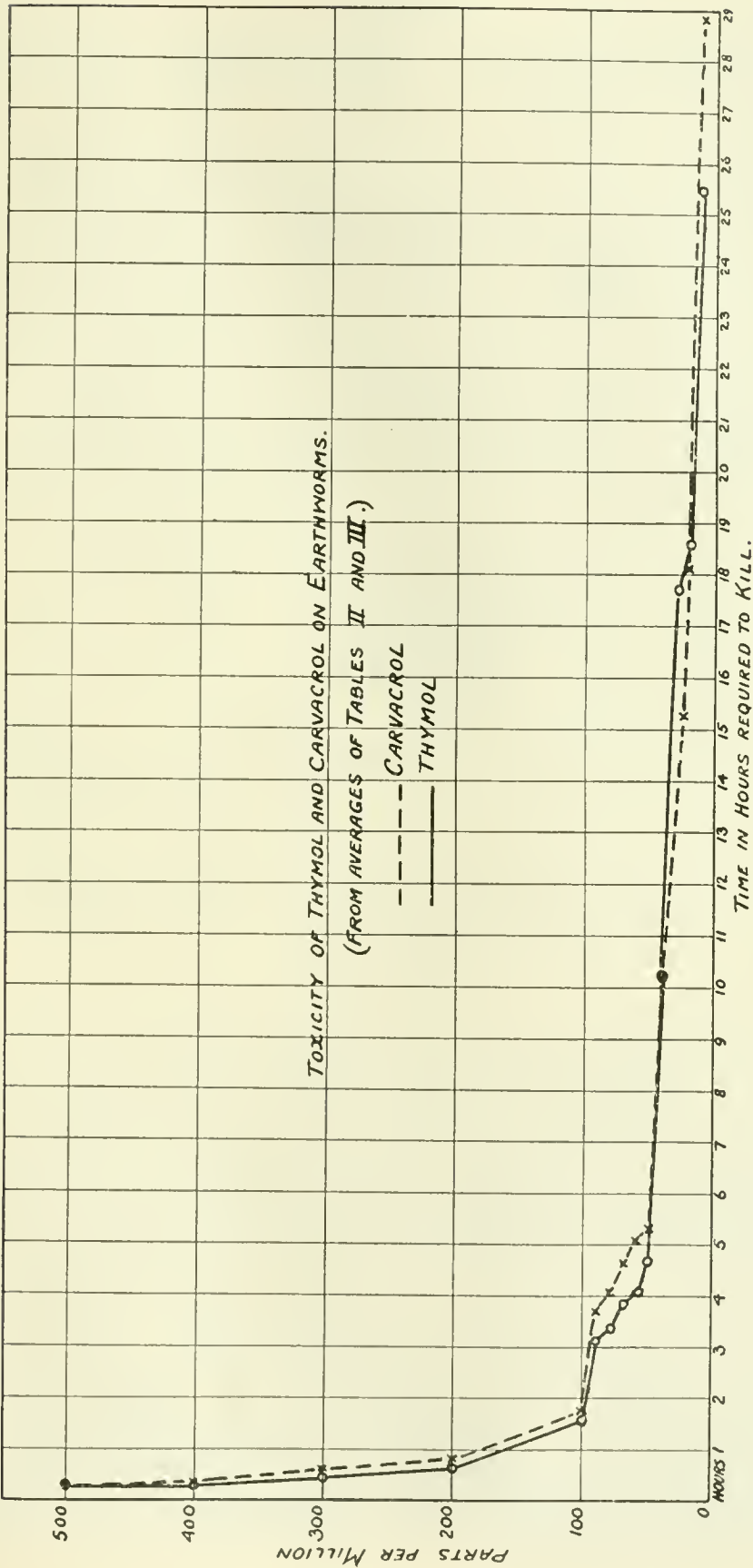


FIG. 1

yellow substance from the middorsal portion of each segment. This gives a beaded appearance along the dorsal surface which is soon thrown off and gives the solution a yellow tint. No evidence has been found which would indicate that this is a waste product, thrown off on account of a stimulating action of the drug, or a protective mechanism designed to neutralize in some way the irritating action of substances with which they may come in contact. With the lower concentration these reactions require more time to develop so that in a solution of 100 parts per million the movements continue for an average of about ninety minutes. Cessation of spontaneous movements is not considered a criterion of the time of death because movements

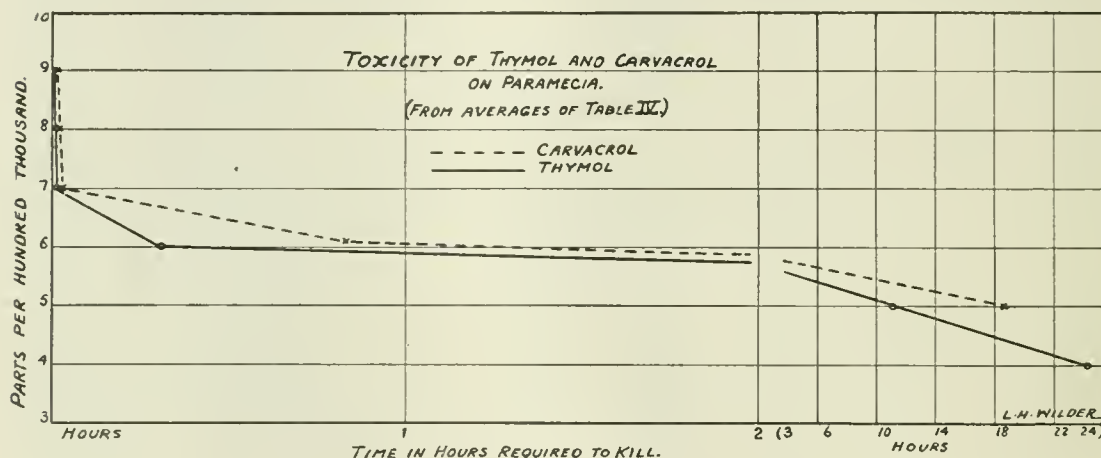


FIG. 2

may still be produced by mechanical stimulation for a somewhat longer time. The exact time at which no reaction can be obtained by mechanical stimulation has been considered as the time of death. The length of time required to produce death by the different concentrations is shown in tables 2 and 3. The corresponding curves plotted from these figures reveal the fact that with most concentrations carvacrol requires slightly more time than thymol in which to produce death. This difference however is so slight that it may possibly lie within the limits of experimental error. An irregularity in the curves for both thymol and carvacrol is shown for concentrations between 50 and 100 parts per million. No explanation can be given at present, for this variation.

TABLE 2
Toxicity of thymol on earthworms

	PARTS PER MILLION																												
	500		400		300		200		100		90		80		70		60		50		40		30		20		10		
	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	
Time in hours and minutes re- quired to kill	0	20	0	25	0	25	0	35	2	0	2	0	2	30	3	30	3	30	3	30	6	0	24	0	25	30	24	0	
	0	20	0	25	0	25	0	35	2	0	2	0	2	30	3	30	3	30	3	30	6	0	5	0	6	30	31	0	
	0	15	0	15	0	25	0	40	1	40	3	30	3	30	4	0	4	30	4	0	5	30	28	0	29	0	24	0	
	0	15	0	15	0	30	0	40	1	40	3	30	3	30	6	0	5	30	5	0	6	30	18	30	18	30	24	0	
	0	17	0	20	0	20	0	30	2	30	2	0	3	30	2	30	4	0	4	0	28	0	18	30	18	30	24	0	
	0	17	0	20	0	30	0	45	1	30	5	30	3	30	2	30	4	0	5	0	5	0	18	30					
	0	20	0	25	0	30	0	50	1	45	3	0	4	0	3	30	6	30	4	15	7	0	18	30					
	0	20	0	25	0	30	0	50	1	45	3	0	4	30	4	30	4	0	4	45	7	0							
						0	45		1	45	1	30	3	0	3	30	4	0	7	0	7	0							
						0	45		1	45	3	30	3	0	5	0	3	20	4	45	17	30							
									2	0	4	0	4	45			3	20			17	30							
									1	15	4	0	4	45			3	30											
									1	15							4	0											
									1	15																			
									1	30																			
									1	15																			
									1	45																			
									1	15																			
									1	15																			
									3	0																			
									3	0																			
	Average....	0	18	0	21	0	31	0	41	1	41	3	8	3	35	3	51	4	8	4	35	10	17	18	43	19	36	25	24

EXPERIMENTS WITH PARAMECIA

For the purpose of supplementing the toxicity experiments with a unicellular type the paramecia was selected. It was soon found by a few preliminary tests that this organism is rapidly killed by 10 parts of the drug per 100,000 parts of dis-

tilled water, while 1 part per 100,000 does not kill. The experiments were therefore confined between these limits of concentration. The method usually followed consisted in arranging nine small test tubes in a rack and placing in them respectively 1, 2, 3, 4, 5, 6, 7, 8, and 9 cc. of a 0.01 per cent solution of the

TABLE 3
Toxicity of carvacrol on earthworms

	PARTS PER MILLION																	
	500		400		300		200		100		90		80		70		60	
	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes	Hours	Minutes
Time in hours and minutes re- quired to kill	0 20	0 25	0 25	0 25	0 45	1 45	2 30	3 0	4 0	4 45	1 15	7 30	23 0	20 30	24 20			
	0 20	0 25	0 30	0 45	1 45	2 30	3 0	4 0	4 45	2 10	6 30	6 30	18 30	31 0				
	0 15	0 15	0 25	0 40	1 40	3 30	3 30	4 30	6 30	5 0	6 30	6 30	19 0	53 0				
	0 15	0 15	0 30	0 40	1 40	3 30	3 30	4 30	6 30	5 0	6 30	18 30	19 0	23 30				
	0 17	0 30	0 40	1 0	1 45	6 0	3 30	4 0	5 30	7 30	5 0	18 30	19 0	23 30				
	0 17	0 30	0 40	1 0	2 25	6 0	7 30	4 45	5 30	4 0	7 0	18 30	19 0	18 30				
	0 20	0 25	0 40	1 0	1 15	4 15	4 0	4 0	3 20	4 0	7 0		19 0					
	0 20	0 25	0 40	1 0	2 0	4 15	4 0	7 0	4 0	6 30	8 0							
			0 45		1 0	3 0	4 0	4 30		5 0	18 30							
			0 45		1 15	3 0	4 0	4 30		7 0	18 30							
					1 15	3 0	4 30			7 0	22 0							
					1 45	3 0				7 0								
					1 10					8 0								
					1 10													
					1 30													
					1 45													
					1 45													
					2 30													
					2 30													
					3 45													
Average	0 18	0 24	0 36	0 51	1 47	3 43	4 3	4 35	5 6	5 20	10 16	15 15	15 19	9 28	58			

drug to be tested. In the same order was added 8, 7, 6, 5, 4, 3, 2, 1 and 0 cc. of distilled water thus each tube contained 9 cc. To each tube was now added 1 cc. of water containing the paramecia which resulted in dilutions, mentioned in the same order, of 1, 2, 3, 4, 5, 6, 7, 8, and 9 parts per 100,000.

TABLE 4

TOXICITY OF THYMOL ON PARAMECIA												TOXICITY OF CARVACROL ON PARAMECIA																									
Parts per 100,000												Parts per 100,000																									
4	5			6			7			8			9			4	5			6			7			8			9								
	Hours	Minutes	Seconds	Hours	Minutes	Seconds	Hours	Minutes	Seconds	Hours	Minutes	Seconds	Hours	Minutes	Seconds		Hours	Minutes	Seconds	Hours	Minutes	Seconds	Hours	Minutes	Seconds	Hours	Minutes	Seconds									
Time in hours, minutes, and seconds required to kill	24	0	0	20	0	0	0	22	0	0	3	0	0	1	0	0	0	30																			
					5	0	0	0	11	0	0	1	30	0	0	30	0	30																			
	20	0	0	18	0	0	0	11	30	0	1	0	0	0	30	0	30																				
								0	30	0	0	2	0	0	40	0	30																				
Average.....																																					
	28	0	0	9	0	0	0	15	30	0	1	0	0	0	44	0	0	25																			

It is necessary especially with the stronger solutions that the time be very carefully observed, when the culture is added and when all paramecia are dead. As soon as the culture was added the tube was inverted once or twice to insure immediate contact of the paramecia with the drug. This was usually accomplished in one or two seconds. A drop of the mixture was immediately placed on a slide under the microscope and carefully observed. Fresh drops were thus examined as rapidly as possible until all paramecia were found to be dead. This procedure was repeated for the various dilutions. The length of time of survival for all dilutions is shown in table 4. From these averages the corresponding curves were plotted. It may be observed that between 5 and 7 parts per 100,000 there is a sharp break in the curve. Only after a day or more were all found to be dead in solutions of less than 5 parts per 100,000 while with 7 parts per 100,000 all are usually dead in two minutes or less. Of course in any given tube some individuals are dead some time before all are dead. It was found that a much more definite end point could be obtained by taking the time at which all were dead instead of when approximately all were dead. The culture of paramecia contained the individuals in numbers such as to permit the dilution as described above and then with a magnification of about 50 times there were usually 4 to 6 in the field of the microscope at any one time. Thus an approximate idea may be gained as to the number of paramecia exposed. The point of most interest at the present time is the fact that there is no striking difference in the toxicity of the two substances for paramecia.

The author wishes to express his thanks to Dr. James E. Benedict of the Smithsonian Institution for assistance in the identification of the earthworms used, and to Professor Carl Voegtlin of this Laboratory for helpful suggestions throughout the work.

CONCLUSIONS

1. The toxicity of thymol and of carvacrol on rabbits is essentially the same.

2. The toxicity of thymol and of carvacrol as tested on paramecia shows no striking difference.

3. Tests on earthworms indicate that the relative anthelmintic values of thymols and carvacrol are practically the same.

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EVIDENCE FOR THE PRESENCE IN DIGITALIS OF A PRINCIPLE THAT IS ELIMINATED RAPIDLY AFTER INTRAVENOUS INJECTION INTO THE CAT¹

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The duration of the cardiac action following the intravenous injection of various digitalis bodies in the cat varies from a period of a day or more after the administration of a nearly fatal dose of strophanthin or of ouabain, to as much as three weeks following the injection of corresponding doses of digitalis or digitoxin (1), but, so far as I know, the literature affords no evidence that any digitalis body exerts a more fleeting action on the cat's heart or that of man than does strophanthin or ouabain.

Hatcher has recently observed (verbal communication) that the cat requires only about half as much of a certain chloroform-soluble fraction of digitalis principles to cause death when it is injected intravenously within a period of a few minutes as it does when the administration is made over a period of four or five hours. This observation is the more surprising in view of the fact that the chloroform-soluble fraction under consideration gives certain of the chemical and biologic reactions characteristic of digitoxin, which behaves in a manner directly opposite to that of this chloroform-soluble fraction with reference to the amounts required to cause death by rapid and slow administration respectively.

Hatcher concluded, therefore, that the chloroform-soluble fraction with which he worked consists of a mixture of digitalis principles, of which at least one is eliminated far more rapidly

¹ Part of the expense of this research has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

after intravenous administration in the cat than are other digitalis principles with which we are acquainted.

It is convenient to speak of the "elimination" of an alkaloidal principle when it has ceased to exert its typical effects, even though we may have no definite proof of its destruction, or excretion from the body, and it is in this sense that the term "elimination" is employed in this paper. The body fluids may dilute an irritant substance or they may neutralize an acid and the animal may recover from the effects of such poisons before they have excreted, but we are accustomed to consider recovery from the effects induced by the administration of a toxic alkaloidal poison as evidence of its destruction, or excretion from the body, and if the administration of an amount of such a poison equal to twice the single fatal dose during a period of several hours or several days is survived, one is justified in supposing that within that period an amount equal to at least a single fatal dose has been eliminated from the body, or rendered incapable of exerting its typical toxic actions. This is especially true when the poison acts directly on the heart causing it to come to a standstill.²

Naturally, this presupposes the absence of any protective mechanism, such as we commonly ascribe to habituation or to the development of tolerance, but such protection is not known to occur with any of the digitalis bodies. Reduction of the activity of the poison by means of the injection of large amounts of fluid, such as Meltzer observed with strychnin (2), does not

² It is well known that poisons which act on several dissimilar structures exert their several actions during varying periods of time, and after a single toxic dose one frequently sees symptoms referable to one structure long after another structure has recovered completely from the effects of the poison. We must suppose, therefore, that such poisons are retained more tenaciously in those organs in which the action (not necessarily the effects) persist than in those which recover more promptly, and since we have no way of knowing when the last traces of poisons are excreted or destroyed, the term "elimination" is of necessity given some elasticity. Hatcher and Eggleston (*J. Pharm. and Exp. Therap.*, 1919, xii, 427) refer to the elimination of a poison from the organ on which its chief, or characteristic, action is exerted, as evidenced by the return of that structure to its normal behavior toward the poison, that is, its complete recovery, as the "essential elimination."

come into consideration here for it is immaterial, so far as the activity of the digitalis bodies is concerned, whether they are administered in fairly dilute, or much more concentrated, solutions, and furthermore, the total amounts of liquids used in most of the experiments did not vary greatly.

Since chemical changes in plant principles are so prone to occur during the process of extraction and purification, it appeared desirable to determine whether digitalis leaves contain any active principle, such as that which Hatcher supposed to be present in his chloroform-soluble fractions and to be rapidly eliminated after intravenous injection in the cat, and if so what percentage of the total activity of the leaf it represents, and also, whether the percentage varies in different specimens of the leaf. The importance of this is obvious in view of the frequent use of the cat for the biologic standardization of digitalis, and the probability that the elimination in man is more or less similar to that in the cat.

It was supposed that it would be a comparatively simple matter to determine whether such a rapidly eliminated digitalis principle exists in the leaf, in view of the relative constancy with which Hatcher obtained evidences of its presence in his chloroform-soluble fractions, and the fair degree of uniformity with which cats behave toward a given digitalis body with a given rate of injection, but the results proved somewhat disappointing in their lack of uniformity, though they do throw some light on the problem.

The failure to obtain more nearly concordant results proved disconcerting at first, but the explanation of the discrepancies is probably to be found in the differences in the behavior of the several digitalis principles, and more especially between that of digitoxin and the rapidly eliminated substance.

When a just fatal dose of digitoxin is injected intravenously into the cat death is delayed during a period varying from a few hours to a day or two, though the heart stops with extraordinary suddenness—often within a few seconds—following the intravenous injection of a massive dose of digitoxin. From this it follows that the more slowly digitoxin is injected intravenously

the smaller is the total amount required with continuous injection to cause death, but, as previously indicated, much more of this rapidly eliminated substance is required apparently when the injection is made slowly and continuously, than when the injection is made rapidly, till death occurs, because elimination removes more of a rapidly eliminated substance during the period of slow injection than during the shorter period required with rapid. Obviously, then, the behavior of a mixture of the two types of digitalis bodies with reference to the amounts required will vary somewhat with the relative amounts of the two which are present. There can be little doubt that this factor enters into the explanation of the variable results observed.

It was therefore sought to compare the maximum toxicity of digitalis (tincture) with rapid injection, usually within a period of twenty minutes to one hour, with the minimum toxicity with slow injection, usually within a period of three to six hours. It was often impossible to maintain the duration of the experiments and the rate of injection as designed, for reasons which become apparent from a study of the problem.

The estimation of the maximum toxicity with rapid continuous injection involves an unavoidable error, since death does not occur until several minutes have elapsed after the injection of the minimal fatal dose. During this interval before death the superfluous amount injected will, of course, depend on the rate of injection, and the more rapid the injection the greater will be this excess over the amount required.

It was sought to minimize this error by injecting a supposedly nearly fatal dose at once, and continuing the injection at a rate somewhat slower than would be required to administer the total dose within the period designed for the experiment with a uniform rate of injection.

The minimum toxicity (within the period allowable for the experiment) was determined by injecting an initial dose designed to be nearly fatal, and after an interval of some hours injecting small amounts at short intervals, or a dilute solution continuously until the heart stopped, or, more accurately, until symptoms indicated that a fatal dose had been administered. In two

experiments in which the minimum toxicity of the English leaf was determined, the "combined ouabain" method described by Hatcher and Brody³ was employed.

In some instances the initial dose proved fatal, and while such experiments are not without value in determining the maximum toxicity of the specimen, they do not indicate just how far the minimum fatal dose was exceeded. When the animal died within a few minutes following such a single injection it was considered an indication that the dose was much above the minimum required for that animal, not necessarily much above the average, since the animal may have been unusually susceptible, but when death was delayed for as much as fifteen to twenty minutes it was accepted as an indication that the dose was not much above the minimum required to cause death.

It will be understood from the foregoing discussion that the failure to cause death during rapid administration with a smaller dose than that required by slower injection does not preclude the presence of at least small amounts of the rapidly eliminated substance, since the unavoidable error inherent in rapid injections, and the increasing action of digitoxin, (if, indeed, there is any free digitoxin present in the leaf) during the longer periods of slow administration, tend to counterbalance the lessened effect of the rapidly eliminated fraction. With some of the specimens used the evidence points unmistakably to the presence of such a rapidly eliminated fraction, in others the results are not conclusive.

The following specimens of digitalis were selected for examination as being fairly representative of the different kinds of leaf commonly used in this country: 1. Allen's English digitalis in no. 60 powder. 2. The leaf of the first year's growth, cultivated in Virginia. 3. Leaf of the first year's growth, cultivated in

³ The "combined ouabain" method of estimating digitalis bodies, consists in determining the difference between the average fatal dose of ouabain for the cat per kilogram of weight, and that required to kill after the previous injection of a dose of any digitalis body. The difference represents the percentage of the fatal dose that is attributable to the digitalis body under investigation. This is illustrated in one of the protocols given. For details of the method see the paper of Hatcher and Brody: *Am. Jour. of Pharmacy*, 1910, lxxxii, 360.

Minnesota. 4. Specimen of fluidextract assayed in 1912 and found to be active. 5. Specimen of fluidextract assayed in 1912 and found to be much less active than the preceding. 6. Specimen of fluidextract recently assayed and found to be active, but which had been submitted for examination, with the complaint that long-continued administration failed to induce the typical therapeutic effects in man.

Tinctures of the leaves were prepared and these were diluted with a small volume of normal salt solution, or weak alcohol, when initial injections of definite amounts were to be made, and with larger amounts of normal salt solution when the injections were to be made slowly and continuously. Fluidextracts were usually diluted with about an equal volume of 20 per cent alcohol for initial injections, and with about 99 parts of normal salt solution for continuous injections.

A protocol, in brief, will be given of an experiment showing the determination of the maximum activity with a shorter period, and of one with a longer period, and the results of the several series will be tabulated. The second experiment detailed was one of the two in which ouabain was used for estimating the percentage of the first dose that remained active.

Test of concentrated tincture of English digitalis; after dilution with normal saline 1 cc. represents 4.76 mgm. of the leaf.

Female cat, gray; weight 2.72 kgm., ether for operation

10.55 to 11.03 a.m. 9.7 cc. per kilogram (representing 46 mg. of digitalis) into the femoral vein; rate of injection slowed.

11.57 a.m. Convulsion and death.

15.8 cc. per kilogram injected (75 mgm.); death in 62 minutes after starting.

Male cat, gray; weight 3.84 kgm.

10.20 a.m. 0.12 cc. concentrated tincture (57 mgm. of digitalis) per kilogram into vein; animal released after closing wound, shows great prostration.

10.27 a.m. Emesis; repeated.

2.53 p.m. Start injection of ouabain solution 1:100,000 slowly into femoral vein.

3.26 p.m. 0.05 mgm. ouabain per kilogram total injected; convulsion and death.

Calculation: Since 0.1 mgm. ouabain is the fatal dose for 1 kgm. weight of cat, 0.05 equals 50 per cent of the fatal dose, indicating that 50 per cent of the fatal dose is due to the 57 mgm. of digitalis previously injected, hence 114 mgm. per kilogram would be required to cause death with this rate of elimination.

Table showing the toxicity of different specimens of digitalis with different intervals following the initial injection and before the death of the animal. The doses are expressed in milligrams of digitalis represented in the tincture or fluidextract administered; the intervals are given in minutes. Fractions are omitted

INITIAL DOSE PER KILOGRAM	INTERVAL	TOTAL DOSE PER KILOGRAM
English leaf		
<i>mgm.</i>	<i>minutes</i>	<i>mgm.</i>
62	7	65
48	33	56
46	62	75
62	299	127
57	306	114
Virginia leaf		
70	20	86
70	20	86
60	23	66
70	23	98
50	27	75
85	41	100
85	56	106
51	58	111
60	70	116
80	306	112
50	384	114
65	397	108
Minnesota leaf		
65	10	65
65	23	76
65	40	70
65	99	97
70	231	90
70	253	82
75	256	86
65	310	91
60	327	103
50	360	98
65	370	86
65	418	99

INITIAL DOSE PER KILOGRAM	INTERVAL	TOTAL DOSE PER KILOGRAM
Fluidextract of 1912		
<i>mgm.</i>	<i>minutes</i>	<i>mgm.</i>
continuous	15	98
110	20	110
continuous	59	116
100	89	110
70	326	142
120*	335	150
70	368	138
70	371	163
70	372	110
Fluidextract (poorer specimen)		
continuous	13	165
160	33	175
160	72	244
160	78	270
160	89	338
160	90	277
100	291	253
130	310	220
130	317	256
130	348	169
100	363	263
Fluidextract (active, but clinically unsatisfactory)		
120†	4	120
120†	10	120
100	29	100
95	85	105
100	91	105
120	323	150
100	359	138
110	368	122
100	368	124

* This animal was evidently tolerant, but the inclusion of the result does not materially affect the average.

† These initial doses were too large, as shown by the rapidity of the onset of death; a dose of 100 mgm. represents the average minimal fatal probably, though a dose of 120 mgm. was survived for some time in another experiment.

Table showing averages of fatal doses of the several specimens with the averages of the shorter and the longer intervals before death

	AVERAGE TIME OF INTERVAL		AVERAGE DOSE PER KILOGRAM
	Interval	Time <i>minutes</i>	
English leaf.....	Short	34	64
	Long	302	121
Virginia leaf.....	Short	26	85
	Long	212	111
Minnesota leaf.....	Short	24	70
	Long	291	92
Fluidextract 1912.....	Short	31	108
	Long	310	137
Fluidextract (poor).....	Short	23	170
	Long	217	254
Fluidextract.....	Short	14	113
	Long	266	124

It may be asked why the number of experiments designed to determine the amounts required to cause death in the shorter and longer periods respectively varies so widely. It was supposed that the duration of the action of the rapidly eliminated substance varies from three to five hours, and that the elimination would be roughly proportional to the interval elapsing after the initial injection and before death. It now seems probable, in the light of further experience, that the rate of elimination varies rather widely in different individuals.

The periods in which death followed after an interval of an hour or less are chosen, somewhat arbitrarily, as the shorter periods, and the remainder for the longer periods. One experiment of sixty-two minutes is included in the shorter series, but the ratio is not altered materially thereby.

One other specimen of digitalis was included in the study but the results with it have been discarded because the maximum toxicity for the shorter period was not determined satisfactorily,

owing to an oversight. One animal—evidently more susceptible than the average—died within a few minutes following a small initial dose, and it would be misleading to accept this dose as representing the average maximum toxicity of the specimen. There was no important difference between the amounts of this specimen required to cause death with intervals varying from 116 to 489 minutes. Though this series is discarded, as stated, the results, so far as they can be accepted, support the results obtained with the other specimens.

It is possible that the difference shown between the amounts of the English specimen required with rapid and slow injection was greater than would be found with a larger series of experiments, but this specimen was very active, as shown by the biologic test as well as by therapeutic use, and it is possible that it contains a large proportion of the rapidly eliminated substance.

There is no doubt that the averages of the amounts given in the shorter periods are somewhat too high for the reasons already stated, i.e., the difficulty of avoiding the injection of too much when the time allowed for the injection is very short. The first experiment with the poor specimen of fluidextract affords an illustration of this. The injection was continuous and a total of 165 mgm. per kilogram of weight were injected within thirteen minutes. It is difficult to avoid the conclusion that all of the drug injected during the last two or three minutes was above the minimal requirement. In the absence of data on which to base a calculation of such errors we are forced to use the figures obtained.

The results with the last fluidextract are somewhat surprising in view of the fact that it was sent to the laboratory for examination because of its failure to induce the characteristic effects in man with long-continued administration. It is more than possible, however, that the rate of elimination in man is quite different from that in the cat. This specimen was used later by Dr. Cary Eggleston, who observed prompt therapeutic effects following its use by the high dosage method, in which, however, the total amount administered to the patient within a period of about twenty-four hours was only about one-eighth of that admin-

istered to one without notable effects over a very much longer period, and which resulted in the complaint just mentioned (oral communication).

These results support the view that the difference between the amounts actually required by rapid and slow injection is greater than that shown in the table, and therefore that the initial doses of 120 mgm. given in the table were too large.

It is regretted that it was impossible to determine the percentages of the rapidly eliminated substance in the several specimens examined, though there is no reason to doubt that a readily eliminated substance, having a true digitalis action exists in the leaf, and one must conclude that it is associated with the chloroform-soluble substance with which Hatcher worked. Its action becomes more prominent when separated from the larger proportion of active principles in the leaf, thus explaining why Hatcher has been able to observe evidences of its presence in his preparation so much more constantly than I have been with the tinctures and the fluidextracts of the leaf.

SUMMARY

Seven specimens of digitalis were examined in order to determine the difference between the amounts required to cause death following the intravenous administration in the cat in short periods as contrasted with the amounts required in longer periods of administration.

Evidence is submitted to show that the total amount of digitalis required in this way to cause death is less when this amount is injected within a few minutes than that required when the administration is prolonged over a period of several hours. This is the opposite of the case with digitoxin of which more is required to cause death promptly than after the lapse of some hours.

The evidence that we are dealing with a body having a true digitalis action is afforded by the fact that it is synergistic with other digitalis bodies, such as ouabain.

The results obtained are interpreted as indicating the presence in the leaf of a digitalis body having a shorter period of cardiac

action in the cat than that of any true digitalis body hitherto studied in this way. This brief period of cardiac action is regarded as evidence for its "elimination" in the sense previously defined.

There is some clinical evidence in support of the view here expressed.

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- (2) MELTZER: Jour. Exper. Med., 1900-1901, v, 643.

A PRELIMINARY PAPER ON THE RELATION BETWEEN THE AMOUNT OF STAINABLE LIPOID MATERIAL IN THE RENAL EPITHELIUM AND THE SUSCEPTI- BILITY OF THE KIDNEY TO THE TOXIC EFFECT OF THE GENERAL ANESTHETICS

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In a paper (1) which appeared in 1913 and was concerned with a study of various diuretics in uranium nephritis, with special reference to the part played by the anesthetic in determining the efficiency of the diuretic, the observation was made that old animals which had been rendered nephropathic by uranium and which were killed without the use of an anesthetic, showed more stainable lipoid material in the tubular epithelium, and especially in the cells of the ascending limb of Henle's loops, than did young animals. The further observation was made that when old animals were anesthetized by Gréhant's anesthetic they became anuric and failed to respond to diuretic solutions, while the young animals anesthetized by the same anesthetic in the same amount per kilogram of body weight, in general remained diuretic and were responsive to these solutions.

In a later paper (2) that was primarily concerned with the pathology induced in the kidneys of dogs of different ages by various anesthetics, the above anatomical findings were confirmed, and in addition the observation was made that a morphine-ether anesthesia in puppies and young animals acutely nephropathic from uranium induced less severe pathological changes in the tubular epithelium of the kidney than did Gréhant's anesthetic, the active anesthetic constituent of which is chloroform.

Histological studies of the kidneys of the animals anesthetized by morphine-ether furthermore showed that the accumulation of lipid material in the renal epithelium of both the young and the old animals was less marked than was the case when Gréhan's anesthetic was employed.

From these observations which have been made in previous papers there would appear to be some relationship between the amount of stainable lipid material in the renal epithelium and the susceptibility of the epithelium to an anesthetic. Furthermore the amount of lipid material which accumulates in the epithelium during an anesthesia is determined in some measure by the age of the animal and the substance employed to induce the anesthesia. It has seemed desirable to extend these observations, for they have to do with a fundamental life state, namely, age, and also with the very practical question of the choice of an anesthetic. For this purpose the following study has been made of two hundred and sixteen dogs that have been employed in different types of experiments in this laboratory during the past six years.

The animals that were used in this investigation varied in age from puppies of one month old to animals in an advanced stage of senility. The oldest animal was eighteen years and two months old. Of this number of animals thirty-two were selected as controls. After a period of observation to determine that the animals were normal in so far as any kidney injury was concerned, the animals were killed without the use of an anesthetic. This group of normal control animals varied in age from one month to eleven years.

One hundred and forty-one normal animals, after a period of observation, were anesthetized by either chloroform or ether. During the period of anesthesia the animals were given intravenously some form of diuretic solution in order to observe the effect of such solutions in increasing the flow of urine or in reestablishing a flow of urine in animals that had been rendered anuric by the anesthetic. This group of normal animals that received an anesthetic varied in age from one month to eight years and two months.

Forty-three of the total number of dogs that are included in this paper were found after a period of study to be naturally nephropathic. Ten of these animals were killed without the use of an anesthetic and served as controls for the naturally nephropathic group. The remaining thirty-three naturally nephropathic animals were anesthetized by either chloroform or ether. As was the case with the normal animals so anesthetized, these naturally nephropathic animals were given during the course of the anesthesia various diuretic solutions. This group of naturally nephropathic animals varied in age from two years and three months to eighteen years and two months.

TECHNIQUE OF THE EXPERIMENTS

All of the animals used in this study were observed for two to six days before any experimental procedure was commenced. During this period the animals were kept in metabolism cages, and excepting the very young puppies, were given from 300 cc. to 500 cc. of water by stomach tube daily. The puppies from one to three months old were allowed to take water from a vessel placed in the cage. All of the animals over two months old were kept on a constant diet which consisted in 100 grams of corn meal bread twice a day. The bread was cooked with a small amount of lard. Each 100 grams of bread contained 30 grams of lean beef. The animals under two months old were fed on a milk mush consisting of corn meal cooked in milk. During this period of observation the urine of the animals was examined qualitatively for albumin, glucose, acetone and diacetic acid. The centrifugalized urines were studied for casts. On two days during a period of observation a renal functional test was made with phenolsulphonéphthalein according to the method devised by Rowntree and Geraghty (3). For a further study of renal function, blood urea determinations were made by the method of Marshall (4) as modified by Van Slyke and Cullen (5), and blood creatinine determinations were made colorimetrically. Observations on the acid-base equilibrium of the blood were made by determining the reserve alkali of the blood by the method of Marriott (6).

At the termination of the control experiments in both normal and naturally nephropathic dogs as well as in these two groups of animals after they had received an anesthetic, kidney tissue was immediately obtained for microscopic study. Such tissue was fixed in 10 per cent formaline, Zenker's fluid and in a solution of corrosive-acetic. Pieces of tissue were also placed in 0.9 per cent sodium chloride solution. This tissue was used within half an hour after it was obtained for frozen sections which were stained for lipoid material. Frozen sections were made at a later period from the formaline fixed tissue and stained for lipoid material by the same method as was employed for the fresh tissue. The observations which are made in this paper concerning the relative amount of stainable lipoid material in the kidneys of animals of different age periods and in the kidneys of animals that have been anesthetized are based on a study of frozen sections from fresh tissue which had not been fixed in formaline. From these studies my observation is in accord with the observation of Bell (7) that tissue may contain much stainable fat when fresh, but may appear fat free when subjected to a period of formaline fixation. Bell considers that droplets of neutral fat are not affected by formaline while droplets of other fatty substances are only stained in fresh tissue. In staining the frozen sections Herxheimer's Scharlach R. method was employed. Herxheimer's stain consists of 2 grams of sodium hydroxide dissolved in 100 cc. of 70 per cent alcohol. To this alkaline alcoholic solution, Scharlach R. is added to saturation. In a recent paper Bullard (8) has shown the great superiority of Herxheimer's Scharlach R. method of staining for lipoid material over the ordinary methods when Scharlach R. or other fat dyes are employed. When formaline fixed tissue or even fresh tissue is stained by the ordinary solutions of Scharlach R. Soudan III, or Nile Blue, much of the lipoid material in the tissue fails to stain. When, however, Herxheimer's Scharlach R. solution is employed, an amount of lipoid material can be microchemically demonstrated which is surprisingly great. Bullard considers the amount of such material that may be demonstrated in tissues when this method is employed to fresh tissue

to be so great that it may be sufficient to account for all of the neutral fat shown by chemical analysis.

At the present time an investigation is in progress in this laboratory which has as its object quantitative determinations of the lipid material in kidney tissue with observations on the relative amount of such material that can be demonstrated by Herxheimer's Scharlach R. method of staining. In the results so far obtained there has been found a definite relationship between the amount of stainable lipid material in the kidney and the percentage of such material obtained by quantitative methods of analysis. These observations will furnish the basis for a future communication.

PART I. CONTROL ANIMALS. THE AMOUNT AND DISTRIBUTION
OF STAINABLE LIPOID MATERIAL IN THE RENAL EPITHELIUM
OF NORMAL ANIMALS OF DIFFERENT AGE PERIODS

Thirty-two normal animals that varied in age from one month to over seven years are included in this group. After a period allowed for normal observations extending over two to four days, during which time the animals were studied by the methods that have been previously outlined, the animals were shot and kidney tissue obtained for histological study. Five experiments have been selected from these normal animals of different age periods and are included in table 1. As will be observed from a study of these five control experiments, selected to represent the group of normal animals of different age periods that were not subjected to the effect of an anesthetic, all of the animals were normal in so far as their kidney function and the acid-base equilibrium of the blood was concerned. The urine has been normal. The elimination of phenolsulphonephthalein in a two-hour period has varied from a maximum output of 81 per cent in a puppy one month old to a minimum output of 61 per cent in an animal over seven years old. Both the blood urea and blood creatinine have shown normal percentages. The blood urea output per 100 cc. of blood has varied from 15 to 18 mgm. The blood creatinine has varied from 1.96 to 2.07 mgm. The reserve alkali of the blood which has been normal has varied from 8.0 to 8.1.

TABLE 1

Normal animals. The relation of the amount and distribution of stainable lipid material in the renal epithelium to the susceptibility of the cells to an anesthetic

NUMBER OF EXPERIMENT	AGE	PHTHALEIN, PER CENT	BLOOD UREA, M.GM. 100 CC.	CREATININE, M.GM. 100 CC.	R. pH	URINE	ANESTHETIC	BLOOD PRESSURE, MM. HG.	URINE PER MINUTE	ONE HOUR AFTER ANESTHETIC				TWO HOURS AFTER ANESTHETIC			AMOUNT AND DISTRIBUTION OF STAINABLE LIPID MATERIAL IN KIDNEY
										Urine per minute	Blood pressure, mm. Hg.	R. pH	Diuretic solution	Urine per minute	Blood pressure, mm. Hg.	R. pH	
1*	1 mo.	81	18	1.96	8.05	Normal	None. Ani- mal killed										Trace. Fine granules con- fined to loops of Henle. None in convoluted tubules
2*	8 mos.	76	16	2.01	8.1	Normal	None. Ani- mal killed										Trace. Fine granules con- fined to loops of Henle. None in convoluted tubules
3*	1 yr.	74	16	2.10	8.1	Normal	None. Ani- mal killed										Trace. Fine granules con- fined to loops of Henle. None in convoluted tubules
4*	2 yrs., 4 mos.	78	19	1.98	8.05	Normal	None. Ani- mal killed										Trace. Fine granules. Oc- casional droplet. Confined to loops of Henle
5*	7 yrs. +	68	15	2.07	8.0	Normal	None. Ani- mal killed										Trace. Fine granules. Oc- casional droplet. Confined to loops of Henle Droplets marked in loops of Henle. Fine granules in convoluted tubule epithe- lium
1	1 mo.	72	15	2.00	8.05	Normal	Ether	118	6	6	138	8.05	Theobromine, 1 per cent. 1 cc. per kgm.	19	140	8.05	Trace. Fine granules con- fined to loops of Henle. None in convoluted tubules

2	8 mos.	78	17 2.13	8.05	Normal	Ether	138	8	5	120	8.05	0.9 NaCl. 20 cc. per kgm.	27	138	8.05	Trace. Fine granules confined to loops of Henle. None in convoluted tubules
3	2 yrs., 7 mos.	76	19 2.16	8.1	Normal	Ether	122	5	3	130	8.0	Theobromine, 1 per cent. 1 cc. per kgm.	6	128	8.0	Droplets marked in loops of Henle. None in convoluted tubule epithelium
4	5 yrs. +	70	13 1.98	8.05	Normal	Ether	132	2	0	138	8.0	0.9 NaCl. 20 cc. per kgm.	0	142	7.9	Droplets marked in loops of Henle. Fine granules in convoluted tubule epithelium
5	8 mos.	76	19 2.08	8.1	Normal	Chloroform	121	2	2	110	8.0	Theobromine, 1 per cent. 1 cc. per kgm.	1	112	7.9	Large droplets in loops of Henle. Fine granules in convoluted tubule epithelium
5, 6	2 yrs., 7 mos.	70	19 2.18	8.05	Normal	Chloroform	118	1	1	120	7.95	0.9 NaCl. 20 cc. per kgm.	0	120	7.9	Large droplets in loops of Henle. Fine granules in convoluted tubule epithelium
7	5 yrs. +	69	14 2.16	8.1	Normal	Chloroform	115	0	0	122	7.9	Theobromine, 1 per cent. 1 cc. per kgm.	0	125	7.85	Marked increase of droplets in loops of Henle and in convoluted tubule epithelium
8	5 yrs. +	72	16 2.09	8.05	Normal	Chloroform	110	0	0	117	7.9	0.9 NaCl. 20 cc. per kgm.	0	118	7.75	Marked increase of droplets in loops of Henle and in convoluted tubule epithelium

* Control.

In this series of control animals the highest elimination of phenolsulphonephthalein has occurred in a puppy one month old. The lowest output of the dye occurred in a dog over seven years old. Observations of this character have not been made in a sufficiently large number of animals to make any statement concerning the influence of the age of a normal animal in determining its ability to eliminate phenolsulphonephthalein.

Following the period of observation on these normal control dogs, the animals were shot and kidney tissue obtained to be stained for lipid material and to be studied by other histological methods. Such tissue has shown no histological evidence of injury. Even in the older animals of the group both the glomeruli and the tubules have appeared normal. There has been no increase in intertubular connective tissue.

Frozen sections stained for lipid material have not only shown a difference in the amount of such material in the renal epithelium, but they have furthermore shown a variation in the location of the lipid material. In young animals from one month to eight months old only a trace of stainable lipid material has been found in the renal epithelium. The material in such young animals has consisted of minute dust-like particles that could be demonstrated in the cells lining the loops of Henle. The cells in the ascending limb of Henle's loop contain more stainable particles than do the cells in the descending limb of the loop. The fat particles have occurred in the peripheral part of the cell cytoplasm. It has been unusual to demonstrate stainable lipid material immediately around the nuclei of these cells. In these young animals I have never observed stainable lipid material in the epithelium of the proximal or distal convoluted tubules. The glomeruli and the intertubular tissue has always been free from such material. In the older normal control animals, between one and seven years old, there has occurred a definite increase in the amount of stainable lipid material in the loops of Henle. In such animals the material is not only present in the form of dust-like particles, but especially in the ascending limb of the loops, the lipid material can be demonstrated in larger drop-like particles. Furthermore, in the very old animals

lipoid material can be demonstrated as very fine stainable particles in the epithelium of the convoluted tubules, especially the proximal convoluted tubules.

From the foregoing study of these thirty-two normal control animals which were not subjected to the toxic effect of an anesthetic, the following conclusions are made.

1. Normal dogs of different age periods show an amount of stainable lipoid material in the renal epithelium which varies with the age of the animal.

2. Very young dogs and puppies show a very small amount of stainable lipoid material, which is confined to the epithelium of Henle's loops and which occurs in the form of dust-like particles.

3. The older normal animals show an increase in stainable lipoid material in the loops of Henle. The lipoid material is found as distinct droplets, as well as in the form of diffuse particles.

4. The oldest animals in the group have not only shown an increase in the amount of stainable lipoid in the cells of the loops of Henle as compared with the puppies and young animals, but they have also shown such stainable lipoid in the form of small particles in the convoluted tubule epithelium.

5. The occurrence of stainable lipoid material varying in amount in the different parts of the tubular unit is not a nutritional disturbance secondary to an injury to the glomeruli with an interference to the renal circulation. The glomeruli are histologically normal.

PART II. NORMAL ANIMALS. THE AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN THE RENAL EPITHELIUM IN ANIMALS OF DIFFERENT AGE PERIODS FOLLOWING AN ANESTHESIA BY ETHER OR CHLOROFORM. THE RELATION OF THE AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL TO THE SUSCEPTIBILITY OF THE KIDNEY TO THE TOXIC EFFECT OF THE ANESTHETIC

One hundred and forty-three normal dogs are included in this group. The animals were anesthetized for a period of two hours by ether or chloroform. Of the number, eight experiments have

been selected from animals of different age periods as representing the typical response of such animals to an anesthesia from ether or chloroform. These experiments are included in table 1, experiments 1 to 8, inclusive. Four of the animals were anesthetized by ether while the remaining four were anesthetized by chloroform.

As will be observed from a study of table 1, experiments 1 to 8, all of the animals in the group were normal in so far as renal function was concerned. The urine was normal and failed to show casts. The elimination of phenolsulphonephthalein has varied in the different animals from an output in two hours of 69 per cent to 78 per cent. The blood urea determinations have varied from a minimum of 13 mgm. to a maximum of 19 mgm. per 100 cc. of blood. Determinations of blood creatinine have varied from 2 to 2.8 mgm. per 100 cc. of blood. The reserve alkali of the blood has varied within the normal from 8.05 to 8.1.

Normal animals anesthetized by ether

A study of the animals of experiments 1 to 4 that were anesthetized by ether shows that immediately following the etherization the blood pressure in the various animals was maintained at a rather high level. The carotid blood pressure varied from a minimum of 118 mm. of mercury to a maximum of 138 mm. of mercury. At this period of the experiments all of the animals were diuretic. The animals of the first two experiments, which were respectively one month and eight months old, were more freely diuretic than were the older animals of experiments 3 and 4. In general the flow of urine per minute in puppies anesthetized by ether has been greater than the flow in older animals. A further study of these experiments shows by the end of the first hour of etherization the puppies and young dogs to be freely diuretic, while the older animals show a decrease in urine formation that is not dependent upon a lowering of blood pressure. In such animals the systemic blood pressure may be higher than it was at the initial reading, which was made at the completion of a satisfactory state of surgical anesthesia.

The puppies of experiments 1 and 2 at the end of the first hour of etherization had a urine flow for the respective animals of six drops and five drops per minute. In the puppy of experiment 1 the blood pressure had increased from 118 to 138 mm. of mercury. In the puppy of experiment 2 the blood pressure had fallen from 138 to 120 mm. of mercury. In the older animal of experiment 3 the flow of urine was reduced from five drops to three drops per minute. The blood pressure, however, during this period was raised from 122 mm. of mercury to 130 mm. of mercury. The old animal of experiment 4 during the first hour of etherization had become anuric. The blood pressure was raised from the normal reading of 132 mm. of mercury to 138 mm. of mercury. The decrease in the flow of urine or the anuria which may develop in a normal animal anesthetized by ether, as has been recently shown (9), is not necessarily dependent upon a fall in systemic blood pressure and an associated inadequate renal circulation.

A study of the changes induced in the acid-base equilibrium of the blood in these normal animals of different ages during an anesthesia from ether shows that the old animals are more susceptible to the anesthetic than are the puppies. The puppies of experiments 1 and 2, at the end of the first hour of etherization, had a reserve alkali of the blood which showed no depletion. The older animals of experiments 3 and 4 after an etherization of this duration showed a depletion in the alkali reserve of the blood. It will be noted that associated with such a depletion in the alkali reserve of the blood the formation of urine by the animals was reduced, and that in the animal of experiment 4 an anuria developed.

At the end of the first hour of the anesthesia the various animals were given diuretic solutions of either 1 per cent theobromine or 0.9 per cent sodium chloride. The flow of urine was increased in all of the animals except the old animal of experiment 4. This animal remained anuric following an intravenous injection of 20 cc. per kilogram of 0.9 per cent sodium chloride solution.

A study of the experiments at the end of the two-hour period of etherization shows the young animals to be freely diuretic.

No change has taken place in the alkali reserve of the blood from the reading which was obtained prior to the anesthetic. The older animals of the group have either remained diuretic with but little change in the alkali reserve of the blood or they have become anuric. Associated with the anuria there has developed a depletion in the alkali reserve of the blood.

The amount and distribution of stainable lipoid material in the kidneys of normal animals of different age periods anesthetized by ether

At the termination of a two-hour period of etherization the kidneys were removed from the animals for study and the experiments terminated.

The kidneys of the puppies and those older animals that remained diuretic and responsive to diuretic solutions and which either showed no depletion in the alkali reserve of the blood, or in which the depletion was late in developing and not below 8.0, have shown only a trace of stainable lipoid material in the ascending limb of Henle's loop. The convoluted tubule epithelium has not shown such stainable material. In such young animals following an etherization for two hours it is difficult to state whether or not the amount of stainable lipoid material is increased when sections from the kidneys of such animals are compared with sections from the kidneys of normal animals of the same age that were not anesthetized and which served as controls for the anesthetized group of animals. The distribution of the small amount of stainable lipoid material is the same in both the control animals and in the animals that have been anesthetized. The stainable lipoid material is confined to the cells of the ascending limb of Henle's loop.

The older animals that have been anesthetized by ether and that have become anuric and non-responsive to diuretic solutions, and have furthermore shown a marked depletion in the alkali reserve of the blood, have shown a distinct increase in the amount of stainable lipoid material in the cells of the ascending limb of Henle's loop. In such animals the lipoid material

occurs in the form of distinct droplets and not in the form of small particles, as is the case for the kidneys of normal control unanesthetized animals of the same age periods. Furthermore, in those old animals in which ether has manifested its toxic effect by rendering the kidney non-responsive to diuretic solutions, stainable lipoid material can be demonstrated in the convoluted tubule epithelium.

The further histological study of kidney tissue from these animals which was stained with haematoxylin and eosin has shown the kidneys of the younger animals to be normal. The older animals which have shown an increase in stainable lipoid material in the epithelium and which became anuric, show in addition to the accumulation of this material, cloudy swelling of the cells of Henle's loops with rarely vacuolation of the cells and an extreme grade of edema with vacuolation in the convoluted tubule epithelium.

When normal animals of different age periods are anesthetized by ether, the factor of the age of the animal shows itself in the amount of lipoid material which accumulates in the renal epithelium, in the severity of the degenerative changes in the epithelium, in the degree of disturbance in the acid-base equilibrium of the blood and in the functional response of the kidney. In puppies and very young animals there is no appreciable increase in the renal epithelium of stainable lipoid material. There is no other histological evidence of injury to these cells. The blood is not depleted of its alkali reserve. Such animals remain diuretic and responsive to diuretic solutions. In old normal animals such a period of etherization induces an increase in the amount of stainable lipoid material in the cells of the loops of Henle and the appearance of such material in the convoluted tubule epithelium. Associated with such an increase in this material, there occurs marked evidence of epithelial degeneration, the alkali reserve of the blood is depleted, and there is a decrease in urine formation or the establishment of an anuria. Such animals are non-responsive to diuretic solutions.

Normal animals anesthetized by chloroform

Seventy-one normal animals of different age periods were anesthetized by chloroform. Experiments 5 to 8, inclusive, are typical of this group of animals and are included in table 1. The animals varied in age from eight months to between five and six years. Following the development of a state of anesthesia from chloroform, the animals in general showed a lower systemic blood pressure than did the etherized animals. Usually the flow of urine per minute is less in animals anesthetized by chloroform than it is in the normal animals that have been anesthetized by ether. The young animals, as represented by experiments 5 and 6, remained diuretic at the completion of a state of surgical anesthesia. The old animals represented by experiments 7 and 8 became anuric. The anuria was not invariably associated with a low systemic blood pressure. The animal of experiment 7 became anuric with a blood pressure of 115 mm. of mercury. Furthermore, at the end of the first hour of the anesthesia the young animals remained diuretic, while the old animals which became anuric with the establishment of a state of anesthesia continued anuric. At the end of the first hour of an anesthesia by chloroform, both the young and the old animals showed a depletion in the alkali reserve of the blood. This depletion is not as marked in the young animals that remain diuretic as it is in the old animals that become anuric. For example, in the animal of experiment 5, eight months old, the reserve alkali of the blood was reduced during the first hour of the anesthesia from 8.1 to 8.0. In the animal of experiment 7, between five and six years old, the reserve alkali was reduced in the same period from 8.1 to 7.9. It will be recalled that when ether was used as the anesthetic the reserve alkali of the blood in the young animals was unaffected by the anesthetic at this stage of the anesthesia, and that the depletion of the alkali reserve of the blood in the old animals from ether was less marked at this period than it is when chloroform is employed.

At the end of the first hour of the anesthesia these normal animals anesthetized by chloroform were given diuretic solutions

of theobromine or sodium chloride solution. Such solutions were either of slight temporary value as diuretics or they had no diuretic effect. In the animal of experiment 5, following the intravenous administration of 20 cc. per kilogram of 0.9 per cent sodium chloride solution, the urine temporarily increased from two to three drops per minute. Within ten minutes the flow of urine was reduced to one drop per minute.

In the older animals in which the anesthesia from chloroform had very greatly reduced urine formation or rendered the animals anuric, the various solutions were of no diuretic value. In experiments 7 and 8 the intravenous use of solutions of theobromine and sodium chloride caused a slight rise in systemic blood pressure. The animals, however, remained anuric.

A study of these representative experiments shows that during the second hour of the anesthesia from chloroform there occurs a marked reduction in the alkali reserve of the blood in the young animals as well as in the old animals. The reduction is much greater in the old animals. In experiment 5, a young animal eight months old, the reserve alkali of the blood was reduced during the second hour of the anesthesia from 8.0 to 7.9. In experiment 8, an animal between five and six years old, the reserve alkali was reduced from 7.9 to 7.75. Associated with such changes in the acid-base equilibrium of the blood the older animals became anuric and non-responsive to diuretic solutions. The younger animals either show a marked reduction in urine formation and are non-responsive to such solutions, or they develop an anuria which is likewise unaffected by diuretic solutions.

The amount and distribution of stainable lipoid material in the kidneys of normal animals of different age periods anesthetized by chloroform

The histological study of tissue from the kidneys of these normal animals of different age periods anesthetized by chloroform shows the following variations in the amount of stainable lipoid material. Tissue from the kidneys of puppies anesthetized

by chloroform have shown a definite increase in the amount of stainable lipoid material in the cells of the loops of Henle when such tissue is compared with kidney tissue from normal unanesthetized animals of the same age which serve as controls and also when a comparison is made with kidney tissue from normal animals of the same age anesthetized by ether. The lipoid material is not in the form of fine granules, as was the case with the normal kidney tissue obtained from unanesthetized animals of this age period and from animals of the same age period anesthetized by ether, but occurs as well-defined droplets which may be so numerous as to obscure the nucleus of the cell and by its presence to so increase the volume of the cells that they encroach upon the lumen of the tubules. In such young animals the convoluted tubule epithelium has rarely shown the presence of stainable lipoid material.

Tissue stained for lipoid material from the kidneys of the older normal animals anesthetized by chloroform has not only shown a marked increase in the amount of such material in the cells of the ascending limb of Henle's loop when compared with kidney tissue from normal control unanesthetized animals and animals anesthetized by ether, but in addition the amount of stainable lipoid material in the convoluted tubule epithelium is very greatly increased. In old animals which became anuric following anesthetization by ether, stainable lipoid material could only be demonstrated in the convoluted tubule epithelium in the form of diffusely arranged particles. In animals of the same age anesthetized by chloroform this material occurs in such amounts that not only are definite droplets found but these droplets may coalesce to form irregular masses within the cells. In animals anesthetized by both ether and chloroform there has been found to exist a definite relationship between the appearance of stainable lipoid material in the convoluted tubule epithelium and the ability of the kidney to form urine.

A further histological study of the kidney tissue from the animals anesthetized by chloroform has shown in the young animals that the epithelium of the loops of Henle and of the convoluted tubules is only slightly injured. These cells show

cloudy swelling without vacuolation. In the older animals, however, the epithelium of both the loops of Henle and the convoluted tubules has shown a marked edema with vacuolation and in the convoluted tubule epithelium an early necrosis.

When animals of different age periods are anesthetized by chloroform the factor of the age of the animal expresses itself in the same manner as in the animals of different age periods anesthetized by ether. The difference in the reaction between the two groups of animals is a quantitative and not a qualitative expression. When chloroform is employed as the anesthetic, there is a greater accumulation of stainable lipid material in the epithelium of the tubules of the kidney, the degenerative changes in these cells are more severe and the disturbance in the acid-base equilibrium of the blood and in the functional response of the kidney is more marked than when ether is the anesthetic of choice.

From the foregoing study of one hundred and forty-three normal animals of different age periods anesthetized by ether or chloroform, the following conclusions are made:

1. Both ether and chloroform are more toxic for the kidneys of old animals than for the kidneys of young animals.
2. The toxicity of these anesthetics for the kidneys of animals at different age periods has shown a definite relationship with the amount of stainable lipid material which may be demonstrated in the kidneys of normal control animals of the same age as the animals that have been anesthetized. Puppies and young animals that show only a trace of stainable lipid material in the loops of Henle and no stainable material of this character in the epithelium of the convoluted tubule, show but little evidence of the toxic effect of the anesthetics. Old animals, in which the amount of stainable lipid material has increased with age of the animal in the loops of Henle and has also made its appearance in small amounts in the convoluted tubule epithelium, are rendered susceptible to the toxic action of these anesthetics; and in such animals the injury to the kidney is marked.
3. The toxic action of the anesthetics upon the kidney is expressed histologically by a further accumulation of stainable

lipoid material in the epithelium of the loops of Henle, and in the old animals by an accumulation of this material in the convoluted tubule epithelium. Furthermore, in such cells in which lipoid material is accumulating other degenerative changes such as cloudy swelling, edema, vacuolation, and necrosis, rapidly develop. These changes are more marked in old animals than in young animals.

The toxic effect of these anesthetics is expressed functionally by a disturbance in the acid-base equilibrium of the blood and by a decrease in urine formation or the establishment of an anuria. These functional changes are also more marked in old animals than in young animals.

4. The investigation finally indicates that in normal animals of the same age chloroform is more toxic for the kidney than ether. This difference in the toxic effect of the two anesthetics for the kidney is shown in that chloroform induces a more marked accumulation of stainable lipoid material in the renal epithelium, that the degenerative changes in these cells are more extensive and of a more severe type, and in a more marked disturbance in the acid-base equilibrium of the blood and a more rapid decrease in urine formation or the development of an anuria.

PART III. NATURALLY NEPHROPATHIC ANIMALS. THE AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN THE RENAL EPITHELIUM OF NATURALLY NEPHROPATHIC ANIMALS BEFORE AND AFTER A PERIOD OF ANESTHESIA BY ETHER OR CHLOROFORM

In a recent paper (10) that was concerned with a study of the naturally acquired chronic nephropathy of the dog, observations were made on the frequency of the occurrence of certain chronic renal injuries in these animals. This pathological study pointed out the very frequent localization of the chronic injury to the glomeruli. The glomerular injury consisted in both a capsular and intracapillary glomerulo-nephropathy. In such kidneys the further observation was made that the glomerular injury was out of proportion to the epithelial injury. These

observations have been recently confirmed by Stengel, Austin, and Jonas (11) in their studies of certain chronic nephropathies of human origin.

When frozen sections of fresh tissue were made from such naturally nephropathic kidneys and stained for lipoid material by Herxheimer's Scharlach R. method, a very large amount of such material could be demonstrated not only in the loops of Henle but also in the convoluted tubule epithelium. More rarely lipoid material could be demonstrated in the endothelium of the capillary loops of the glomeruli and also in the epithelial lining of these structures.

In a second (12) study of these naturally nephropathic animals that was concerned not only with the pathology of the kidney but also with its physiological response after the animal had been rendered acutely nephropathic by uranium or an anesthetic, the observation was made that such kidneys with a chronic glomerular injury were highly susceptible to the toxic action of both uranium and an anesthetic. Such substances very rapidly rendered these animals anuric and they failed to respond to different diuretic solutions. This decrease in the functional capacity of the kidney was not due to the development of any acute degenerative changes in the glomeruli or to any lack of peripheral response on the part of the vascular mechanism of the kidney when this response was studied by vasoconstrictors as epinephrin and vasodilators as caffeine. The anatomical basis that differentiated a functionally active kidney from a functionally inactive organ consisted in the degree of preservation of the renal epithelium.

With these previous observations in mind the following study has been undertaken of forty-three naturally nephropathic animals with the object of ascertaining if there is any relationship between the amount of stainable lipoid material which occurs in the renal epithelium of such animals and the susceptibility of the kidneys to the toxic action of ether and chloroform.

Control naturally nephropathic animals. The amount and distribution of stainable lipoid material in the renal epithelium of such animals that have not received an anesthetic

Ten of the forty-three naturally nephropathic animals have been selected for these observations and serve as control animals for the group. After a period of study to determine that the animals were naturally nephropathic they were shot and the kidneys studied histologically by the methods previously described. The findings in three of these animals, which are typical for the group, are included in table 2. In this table the age of the respective animals has been indicated. This factor, the age of the animal, cannot be considered as an entity in these experiments, for the existence of the chronic nephropathy irrespective of the age, as will be seen, increases the susceptibility of the kidney to injury.

A study of the control animals of experiments 1, 2, and 3, table 2, shows that all of the animals have a low output of phenolsulphonephthalein. The elimination of the dye has varied from the low maximum output of 47 per cent to 36 per cent. Two of the animals showed a slight retention of blood urea, 20 and 21 mgm. per 100 cc. of blood. The blood creatinine in the different animals has varied from 1.97 mgm. to 2.71 mgm. per 100 cc. of blood. It will be noted that the animal of experiment 3, with a blood urea retention of 21 mgm., also had the highest retention of blood creatinine, 2.71 mgm. per 100 cc. of blood.

All of the ten control naturally nephropathic animals showed a normal alkali reserve of the blood which varied from 8.0 to 8.05. The urine of all the animals showed constantly or intermittently a trace of albumin and a few hyaline or finely granular casts. During the period of four days allowed for observation the dogs were kept on the regular diet that has been used in all of the experiments. At the end of this period the animals were shot.

Fresh tissue from the kidneys of these animals when stained for lipoid material showed a marked accumulation of such

material in both the descending and ascending limbs of Henle's loops. In these locations the lipid material is in the form of large droplets, which may obscure the nuclei and outline of the cells. Furthermore, stainable lipid material in the form of granules and formed droplets is demonstrable in both the proximal and distal convoluted tubule epithelium. This latter observation is of much significance. In the large number of normal animals in which fresh kidney tissue was stained for lipid material by Herxheimer's Scharlach R. method, I was unable to demonstrate such material in the convoluted tubule epithelium, except as fine granules in the oldest of the normal animals. It would appear in these naturally nephropathic animals that the glomerular injury, plus the age of the animal, effects such a nutritional disturbance in the tubular unit that not only is lipid material in considerable amount deposited in the cells of the loops of Henle, but there also occurs a deposition of such material in the convoluted tubule epithelium. The amount of stainable lipid material in the renal epithelium of naturally nephropathic animals is much greater than can be microchemically demonstrated in the kidneys of normal animals of even a greater age limit.

The histological study of kidney tissue from these control animals, stained with haematoxylin and eosin has shown a chronic capsular and intracapillary injury to the glomeruli which is out of proportion to the epithelial injury. The tubular epithelium which has shown the presence of lipid material has been swollen from the mechanical presence of this material, and in addition the convoluted tubule epithelium has shown an early cloudy swelling.

Naturally nephropathic animals. The amount and distribution of stainable lipid material in the renal epithelium following an anesthesia by ether or chloroform

Thirty-three naturally nephropathic animals were anesthetized for two hours by ether or chloroform. The results obtained in ten representative experiments in which five of the animals received ether and five chloroform have been included in table 2.

TABLE 2

Naturally nephropathic animals. The relation of the amount and distribution of stainable lipid material in the renal epithelium to the susceptibility of these cells to an anesthetic

NUMBER OF EXPERIMENT	AGE	PHTHALEIN, PER CENT	BLOOD UREA, MGM. 100 cc.	CREATININE, MGM. 100 cc.	R. pH	URINE	ANESTHETIC	BLOOD PRESSURE, MM. Hg	URINE PER MINUTE	ONE HOUR AFTER ANESTHETIC				TWO HOURS AFTER ANESTHETIC			AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN KIDNEY
										Urine per minute	Blood pressure, mm. Hg	R. pH	Diuretic solution	Urine per minute	Blood pressure, mm. Hg	R. pH	
1*	3 yrs. +	43	20	2.15	8.05	Albumin. casts	None. Ani- mal killed	113	4	3	121	8.0	0.9 NaCl. 20 cc. per kgm.	11	118	7.95	Droplets in loops of Henle. Granular lip- oid material in con- volved tubules
2	2 yrs., 3 mos.	47	16	1.97	8.0	Trace of al- bumin. casts	None. Ani- mal killed										Droplets in loops of Henle. Granular lip- oid material in con- volved tubules
3*	4 yrs. +	36	21	2.71	8.05	Trace of al- bumin. casts	None. Ani- mal killed										Droplets in loops of Henle. Granular lip- oid material in con- volved tubules
1	4 yrs. +	52	14	2.10	8.05	Trace of al- bumin. casts	Ether	113	4	3	121	8.0	0.9 NaCl. 20 cc. per kgm.	11	118	7.95	Droplets and masses in loops of Henle. Gran- ular lipid material in convolved tubules
2	7 yrs. +	40	19	2.04	8.05	Trace of al- bumin. casts	Ether	116	0	0	116	7.9	0.5 Theocin. 0.5 cc. per kgm.	0	120	7.85	Droplets and masses in loops of Henle. Drop- lets of lipid material in convolved tubules

3	5 yrs., 7 mos.	52	16 2.10	8.05	Trace of al-bumin. casts	Ether	120	0	0	125 7.95	Theobromine, 1 per cent. 1 cc. per kgm.	0	1147.85	Droplets and masses in loops of Henle. Droplets of lipid material in convoluted tubules
4	3 yrs. +	43	14 2.15	8.0	Trace of al-bumin. casts	Ether	110	1	0	1147.95	0.9 NaCl. 20 cc. per kgm.	7	1187.95	Droplets and masses in loops of Henle. Granular lipid material in convoluted tubules
5	4 yrs., 1 mos.	48	19 2.31	8.1	Trace of al-bumin. casts	Ether	120	0	0	1207.95	Theobromine, 1 per cent. 1 cc. per kgm.	0	1187.85	Droplets and masses in loops of Henle. Droplets of lipid material in convoluted tubules
6	5 yrs. +	43	16 2.33	8.0	Trace of al-bumin. casts	Chloroform	110	0	0	1007.9	Theocin, 0.5 per cent. 0.5 cc. per kgm.	0	1057.8	Droplets and fused masses of lipid material in loops of Henle and convoluted tubules
7	2 yrs., 6 mos.	51	16 2.08	8.05	Trace of al-bumin. casts	Chloroform	100	0	0	957.85	0.9 NaCl. 20 cc. per kgm.	0	1217.75	Droplets and fused masses of lipid material in loops of Henle and convoluted tubules
8	2 yrs. +	58	20 2.17	8.1	Trace of al-bumin. casts	Chloroform	101	0	0	927.9	Theobromine, 1 per cent. 1 cc. per kgm.	0	957.8	Droplets and fused masses of lipid material in loops of Henle and convoluted tubules
9	7 yrs. +	38	19 2.12	8.05	Trace of al-bumin. casts	Chloroform	115	0	0	1057.9	0.9 NaCl. 20 cc. per kgm.	0	1007.85	Droplets and fused masses of lipid material in loops of Henle and convoluted tubules
10	6 yrs. +	41	20 2.36	8.0	Trace of al-bumin. casts	Chloroform	118	0	0	1077.8	Theobromine, 1 per cent. 1 cc. per kgm.	0	1027.7	Droplets and fused masses of lipid material in loops of Henle and convoluted tubules

As will be observed from a study of the findings in these animals prior to the use of an anesthetic, all of the animals were naturally nephropathic. In so far as the functional response of the kidney is concerned, these animals show a functional response which is comparable to the response obtained in the control naturally nephropathic animals. Eighteen of the thirty-three naturally nephropathic animals were anesthetized by ether. The remaining fifteen animals were anesthetized by chloroform.

Naturally nephropathic animals anesthetized by ether

A study of the experimental findings in the five animals anesthetized by ether and presented in table 2, shows that at the completion of the anesthesia the animals had a systemic blood pressure varying from a minimum of 110 mm. of mercury to a maximum pressure of 120 mm. of mercury. At this early stage of the experiments three of the animals had become anuric. The remaining two animals were forming respectively four drops and one drop of urine per minute.

By the end of the first hour of the experiments only one of the animals, experiment 1, remained diuretic. The systemic blood pressure in both the anuric animals and in the diuretic animal was as high or higher than at the commencement of the experiment. At this stage of the experiments the reserve alkali of the blood in all of the animals had been depleted. The depletion was less marked in the animal of experiment 1 that remained diuretic.

Following these observations the animals were given intravenously diuretic solutions of either theobromine, theocin or isotonic sodium chloride solution. The animal of experiment 1, which had remained diuretic and showed a reserve alkali depletion from the normal of 8.05 to 8.0, responded to a solution of sodium chloride. The urine increased from three drops per minute to eleven drops. The animal of experiment 4, which had become anuric and also showed only a slight depletion in the alkali reserve of the blood, responded to a solution of sodium chloride with a reestablishment in the flow of urine to seven drops per minute. The remaining animals, experiments 2, 3

and 5, showed no diuretic effect from solutions of theobromine or theocin. At the end of these experiments the alkali reserve of the blood had undergone a severe depletion to the low reading of 7.85.

The amount and distribution of stainable lipoid material in the renal epithelium of naturally nephropathic animals anesthetized by ether

In all of the naturally nephropathic animals anesthetized by ether there occurred a distinct increase in the amount of stainable lipoid material in the renal epithelium as compared with the kidneys of the naturally nephropathic animals that were used as controls and not anesthetized. The amount of lipoid material in the loops of Henle was especially increased, and in the animals that became anuric there was a marked accumulation of lipoid material in the convoluted tubule epithelium. Such cells furthermore showed edema with vacuolation of the cytoplasm, and in many of the cells a disappearance of the nuclei.

The naturally nephropathic animals which remained diuretic and responsive to diuretic solutions following anesthetization by ether showed the same type of epithelial injury, but the changes in the epithelium were not so far advanced. The amount of lipoid material in the convoluted tubule epithelium of such animals is smaller in amount, more diffuse in distribution, and does not occur as droplets, as is the case in the animals that became anuric.

Naturally nephropathic animals anesthetized by chloroform

Fifteen naturally nephropathic animals were anesthetized by chloroform. The results in five of these animals are given in detail in table 2.

Following the establishment of a state of surgical anesthesia by chloroform, the carotid blood pressure in the various animals varied from 100 mm. of mercury to 118 mm. of mercury. At this early stage of the experiments the animals became acutely anuric and remained so throughout the two-hour period of the

anesthesia. In such animals becoming acutely anuric there was associated with the development of this state a rapid depletion in the alkali reserve of the blood. This depletion was more rapid and was also more marked in animals anesthetized by chloroform than in those anesthetized by ether. In the animal of experiment 8 the reserve alkali was depleted within an hour from the normal of 8.1 to 7.9. At the end of the second hour of the anesthesia the reserve alkali was 7.8. In the animal of experiment 10, at the end of the first hour of the anesthesia the reserve alkali was reduced from the normal of 8.0 to 7.8, and by the end of the second hour the depletion had reached the very low reading of 7.7.

At the end of the first hour of the anesthesia from chloroform these naturally nephropathic animals were given intravenously diuretic solutions in the form of theobromine, theocin and isotonic sodium chloride solution. These solutions were of no diuretic value in any of the animals.

At the termination of the experiments the blood pressure varied in the different animals from a minimum of 95 mm. of mercury to 121 mm. of mercury.

The kidneys of naturally nephropathic animals anesthetized by chloroform are more susceptible to the toxic effect of this anesthetic than are the kidneys of such animals anesthetized by ether. This toxic action is expressed functionally by the chloroform inducing a more marked disturbance in the acid-base equilibrium of the blood and by the anesthetic rendering the animals anuric.

The amount and distribution of stainable lipoid material in the renal epithelium of naturally nephropathic animals anesthetized by chloroform

The most striking change in the pathology of the naturally nephropathic kidney following a period of anesthesia by chloroform is the extensive accumulation of stainable lipoid material in the renal epithelium and the accumulation to a less extent of such material in the glomerular capillaries and in the epi-

thelium of the capsule. In the loops of Henle, and especially in the ascending limb of the loop, the droplets of fat have increased to such an extent that they fuse to form masses which may entirely obscure the cell. The cells in these tubules are increased in size from the accumulation of such lipid material and also from edema of the cell cytoplasm.

The amount of stainable lipid material in the proximal and distal convoluted tubule epithelium has shown a marked increase, not only over the amount of such material which could be demonstrated in such tubules in normal control naturally nephropathic animals, but the amount is in excess of the accumulation of such material which occurs in the epithelium of the kidney of naturally nephropathic animals anesthetized by ether.

In the convoluted tubule epithelium the lipid material occurs in two forms: diffusely scattered granules and as droplets which have apparently arisen from a fusion of the smaller particles. Frequently large masses of lipid material are found in these cells and in such areas the structure of the cell is obscured.

The epithelium of the convoluted tubules in addition to showing the marked accumulation of lipid material also shows a marked degree of edema, vacuolation, and early necrosis.

Degenerative changes of this character as well as the accumulation of lipid material are more marked and extensive in naturally nephropathic animals anesthetized by chloroform than in such animals when ether is employed as the anesthetic agent.

GENERAL CONCLUSIONS

1. In normal animals of different age periods there has been demonstrated a larger amount of stainable lipid material in the kidneys of old dogs than in the kidneys of puppies and young dogs. Furthermore, the distribution of such material varies with the age of the animal. In puppies such lipid material is confined to the cells of the loops of Henle. In very old animals stainable lipid material can be demonstrated in very small amount in the convoluted tubule epithelium.

2. When normal animals of different age periods are anesthetized by either chloroform or ether, there is found to exist a definite relationship between the toxicity of the anesthetic for the renal epithelium and the age of the animal.

3. These anesthetics are more toxic for the kidneys of old animals than they are for the kidneys of puppies and young animals. This variation in toxicity is expressed histologically by more extensive degenerative changes in the kidneys of old animals and by a more marked decrease in the functional response of the kidneys of such animals.

4. Chloroform has been found to be more toxic for the kidneys of both old and young normal animals than ether. The former anesthetic induces more evidence of epithelial degeneration in the kidney.

5. When kidney tissue of normal animals of different age periods that have been anesthetized by chloroform or ether is stained for lipoid material and studied histologically, there has been found to occur a greater accumulation of such material in the kidneys of those animals anesthetized by chloroform than in the kidneys of the animals anesthetized by ether.

The amount of stainable lipoid material in the renal epithelium of animals of different age periods determines the susceptibility of the kidney to the anesthetic, and the anesthetic employed plus the age of the animal furthermore determines the amount of such material which accumulates in the epithelium during a period of anesthesia.

6. When naturally nephropathic animals are killed without the use of an anesthetic and the kidneys studied histologically, the animals have been found to have a glomerulo-nephropathy with but slight histological evidence of epithelial injury.

When fresh tissue from such kidneys is stained for lipoid material, the epithelium of the loops of Henle and the convoluted tubule epithelium is found to contain stainable lipoid material which is much in excess of that which can be demonstrated in the epithelium of normal animals. A severe injury to the glomerulus is apparently first expressed, in so far as the renal epithelium is concerned, by such a disturbance in the metabolism

of these cells that lipid material accumulates far in excess of the amount normal for the cells.

7. When such naturally nephropathic animals are anesthetized by chloroform or ether they show an increased susceptibility to the anesthetic which is characterized locally in the kidney by a great increase in the amount of stainable lipid material and by the development of marked degenerative changes in the tubular epithelium. The severity of the degenerative changes and the accumulation of stainable lipid material are far in excess of similar changes induced in the renal epithelium of normal animals by the same anesthetics during a period of anesthesia of the same duration.

8. The general toxic effect of ether and chloroform in both normal and naturally nephropathic animals is shown by a disturbance in the acid-base equilibrium of the blood. The depletion in the alkali reserve of the blood which follows the use of these anesthetics is more marked in old animals than in young animals. Furthermore, the use of chloroform effects a greater disturbance in the acid-base equilibrium of the blood in animals of different age periods than does ether. Finally, the naturally nephropathic animals show even a greater depletion in the alkali reserve of the blood following the use of the anesthetics than do the very old normal animals.

9. In conclusion, the results of the investigation indicate a definite relationship between the amount of stainable lipid material in the renal epithelium of both normal and naturally nephropathic animals and the susceptibility of this epithelium to the toxic effect of both ether and chloroform.

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EXPLANATION OF FIGURES

FIG. 1. MICROPHOTOGRAPH FROM A FRESH FROZEN SECTION OF THE KIDNEY OF THE NORMAL CONTROL ANIMAL OF EXPERIMENT 2, TABLE 1

The section was stained by Herxheimer's Scharlach R. method for lipoid material. The animal was a puppy eight months old. The lipoid material appears as dark granules, few in number, confined to the epithelium of the loops of Henle.

FIG. 2. MICROPHOTOGRAPH FROM A FRESH FROZEN SECTION OF THE KIDNEY OF THE NORMAL CONTROL ANIMAL OF EXPERIMENT 5, TABLE 1

The section was stained by Herxheimer's Scharlach R. method for lipoid material. The animal was over seven years old. The amount of lipoid material, which appears as dark granules and droplets, is distinctly increased in amount over that which could be demonstrated in the renal epithelium of the younger normal control animal as shown in figure 1. The lipoid material in this old normal control animal is largely confined to the epithelium of the loops of Henle. Here it appears as droplets. Small granules of this material also appear in the convoluted tubule epithelium.

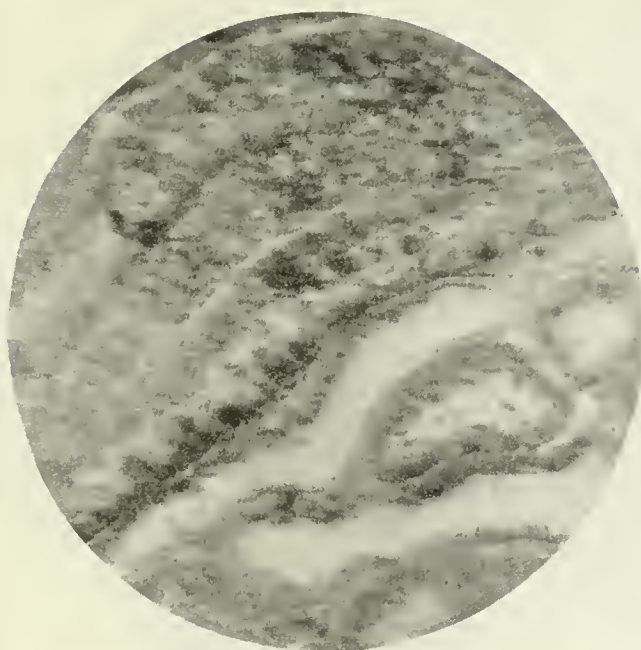


FIG. 1

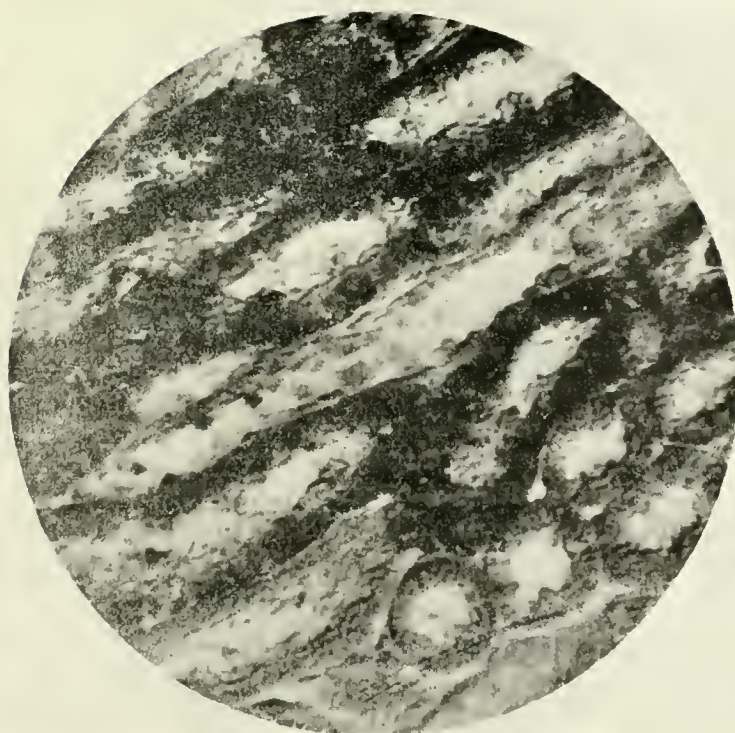


FIG. 2

FIG. 3. MICROPHOTOGRAPH FROM A FRESH FROZEN SECTION OF THE KIDNEY OF THE ANIMAL OF EXPERIMENT 4, TABLE 1

The section was stained by Herxheimer's Scharlach R. method for lipoid material. The animal was five years old. Following an anesthesia by ether the animal became anuric. The stainable lipoid material is greatly increased in amount and is far in excess of the amount of such material that could be demonstrated in a normal control animal of even an older age period. Fig. 2. The lipoid material is in the form of granules and droplets that are beginning to fuse to form larger and less discrete areas. The lipoid material appears in the figure as dark grey or black areas which outline the loops of Henle. The convoluted tubule epithelium contains stainable lipoid material in much smaller amount and in the form of more diffusely arranged particles.

FIG. 4. MICROPHOTOGRAPH FROM A FRESH FROZEN SECTION OF THE KIDNEY OF THE ANIMAL OF EXPERIMENT 10, TABLE 1

The section was stained by Herxheimer's Scharlach R. method for lipoid material. The animal was over six years old. Following an anesthesia by chloroform the animal rapidly became anuric. The stainable lipoid material, which appears as black masses in both the epithelium of the loops of Henle and the convoluted tubule epithelium, is very greatly increased over the amount of such stainable lipoid material which can be demonstrated in the kidneys of normal control animals of the most advanced age. In practically all of the tubules the lipoid material has accumulated to such an extent that it no longer appears as discrete droplets, but the droplets have fused to form masses.

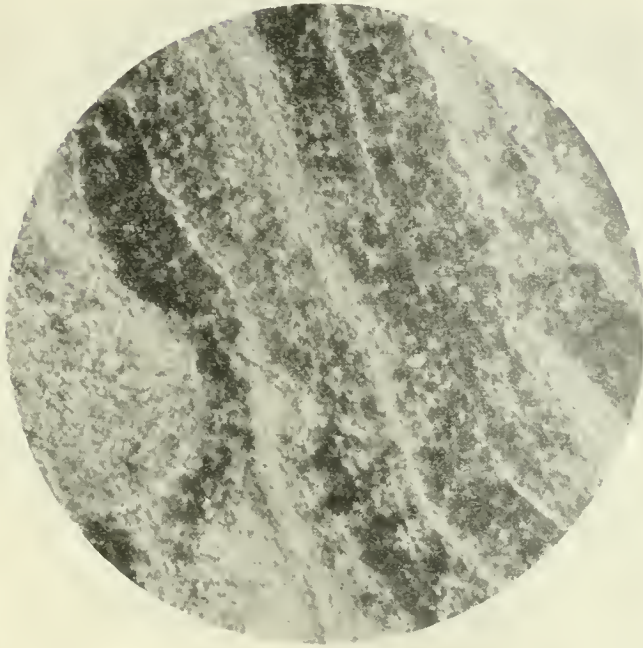


FIG. 3

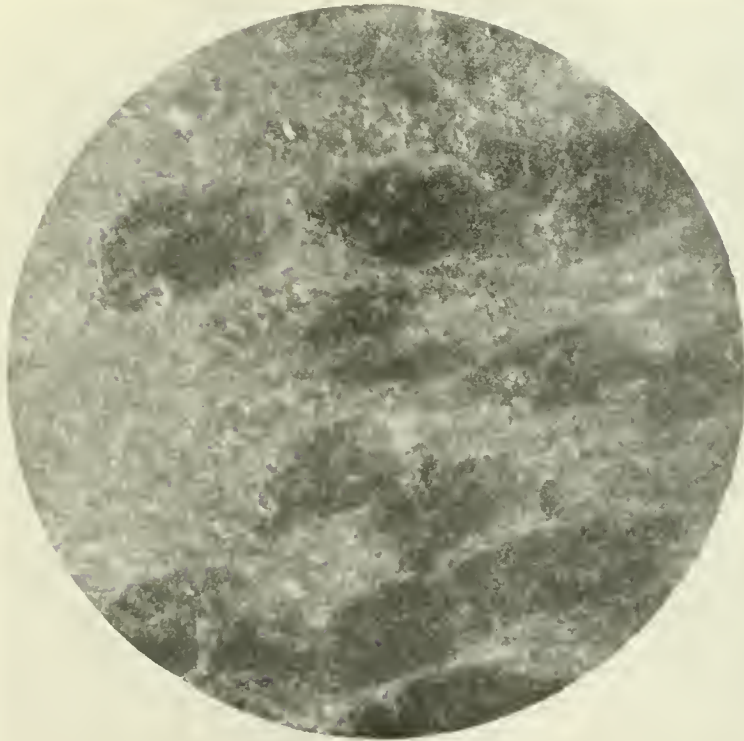


FIG. 4

FIG. 5. COLORED MICROPHOTOGRAPH FROM A FRESH FROZEN SECTION OF THE KIDNEY OF THE CONTROL NATURALLY NEPHROPATHIC ANIMAL OF EXPERIMENT 1, TABLE 2

The animal was killed without the use of an anesthetic. The section was stained for lipoid material by Herxheimer's Scharlach R. method and counter stained with Mayer's Haemalum. The animal had a chronic glomerular nephropathy. In the upper left hand quadrant of the figure is shown a portion of a glomerulus in the cellular stage of a fibrosis. The nuclei of the capsule are prominent and hyperchromatic. The injury to the tubular epithelium is slight and out of proportion to the degree of glomerular injury. Lipoid material, which stains red, is seen in considerable amount in the epithelium of the loops of Henle and also to a less extent in the convoluted tubule epithelium. Such material appears as granules and droplets.

FIG. 6. COLORED MICROPHOTOGRAPH FROM A FRESH FROZEN SECTION OF THE NATURALLY NEPHROPATHIC ANIMAL OF EXPERIMENT 10, TABLE 2.

The section was stained for lipoid material by Herxheimer's Scharlach R. method and counter stained with Mayer's Haemalum. The animal had a chronic glomerulo-nephropathy. The animal was anesthetized with chloroform. In the upper right hand quadrant of the figure is shown a portion of a glomerulus in an advanced stage of fibrosis. The stainable lipoid material in the glomerulus appears as a red, granular deposit. As a result of the anesthesia by chloroform the stainable lipoid material in the renal epithelium has very greatly increased over the amount which could be demonstrated in any of the control naturally nephropathic animals which were killed without an anesthetic. Not only is the amount of stainable lipoid material greatly increased in the epithelium of the loops of Henle, but in the convoluted tubule epithelium this material shows in the form of large masses that obscure the cells and stains red. In some of the tubules the red stained droplets of lipoid material have not completely fused. In other tubules this material appears as a structureless mass. The tubules in which such an accumulation of lipoid material has occurred are in an advanced stage of edema and necrosis. The nuclei have disappeared.

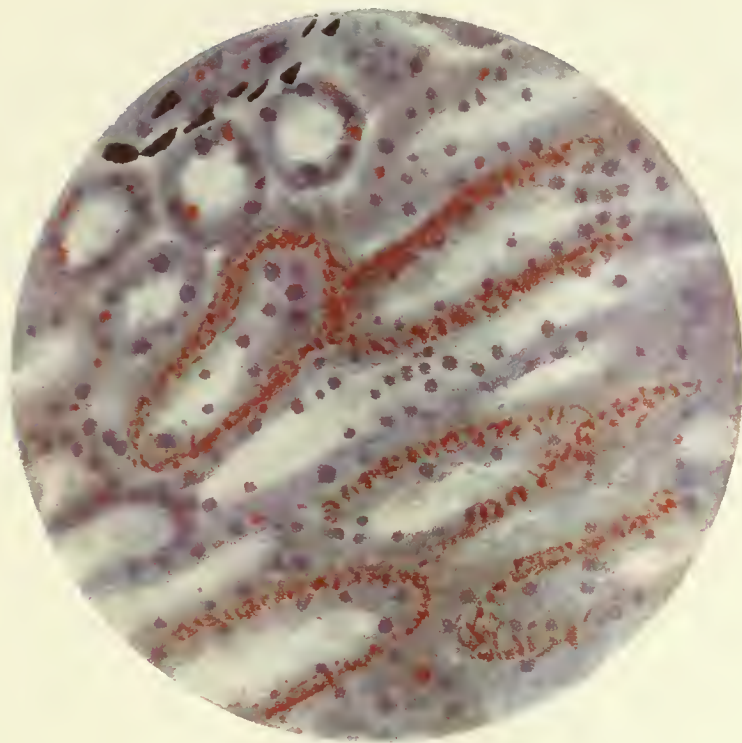


FIG. 5

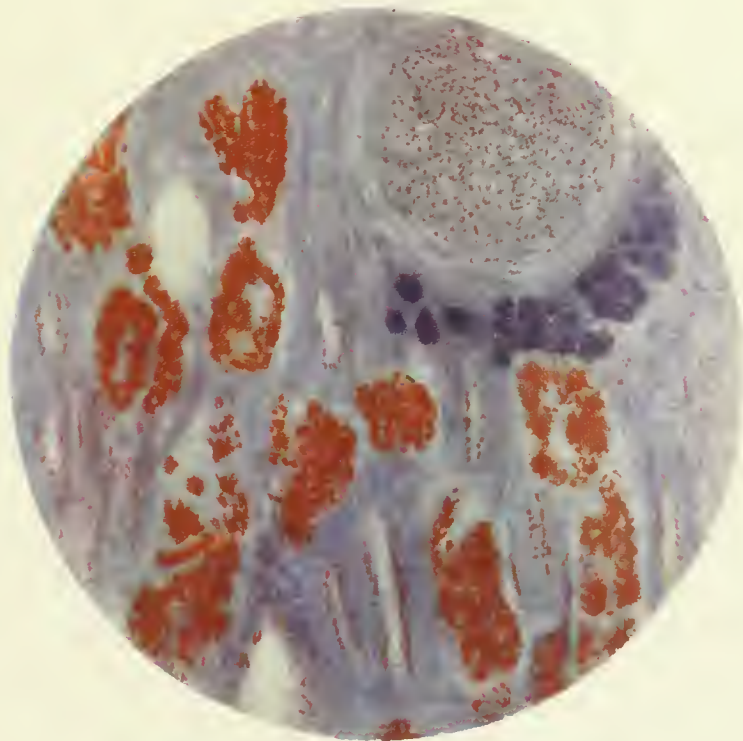


FIG. 6

SCIENTIFIC PROCEEDINGS OF THE AMERICAN
SOCIETY FOR PHARMACOLOGY AND
EXPERIMENTAL THERAPEUTICS

TWELFTH ANNUAL MEETING, HELD AT CHICAGO,
DECEMBER 28-29-30TH, 1920

Edited by the Secretary, Dr. E. D. Brown

Further Studies on Saligenin: Its Mercury Derivative and Allied Compounds. ARTHUR D. HIRSCHFELDER, MERRILL C. HART (by invitation), AND FRANK J. KUCERA (by invitation). From the Department of Pharmacology, University of Minnesota.¹

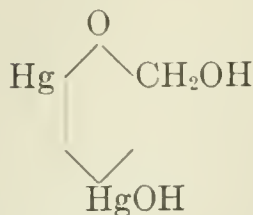
In a previous paper Hirschfelder, Lundholm and Norrgaard have shown that out of a considerable number of phenyl alcohols, saligenin (salicyl alcohol C_6H_4OH (1) CH_2OH (2)) proved to have the best and surest local anesthetic; that it was not irritating to tissues; and that it is suitable to surgical anesthesia, surpassing benzyl alcohol in this respect. Further studies have confirmed this observation and have demonstrated that not only minor operations, but herniotomies, thyroidectomies and laparotomies can be performed satisfactorily under infiltration anesthesia with two per cent saligenin. Hirschfelder and Wynne have shown that 4 per cent saligenin solutions also produce satisfactory anesthesia for cysto-copy of the female urethra, several patients finding it as satisfactory as 10 per cent cocaine. Four and 10 per cent saligenin solutions have been found to give satisfactory anesthesia for cystoscopy in the male. The toxicity is less than one-twentieth that of cocaine.

Beta-hydroxyethyl-aniline ($C_6H_5NHCH_2CH_2OH$) possesses some local anesthetic properties but considerably less than saligenin.

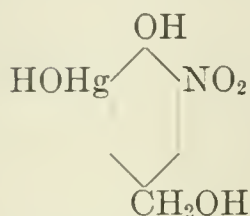
Saligenin and other phenylcarbinols, homosaligenin, and piperonyl alcohol as well as p-hydroxy meta-aminophenyl carbinol (Edinol) are very mild antiseptics, so that two per cent solutions require from one half to one hour to kill staphylococcus, streptococcus, *Bacillus coli*, pneumococcus and gonococcus in bouillon.

¹ The researches reported in this investigation were rendered possible by a grant from the United States Interdepartmental Social Hygiene Board, for the discovery of better medical measures for the treatment of venereal diseases.

We have synthesized a mercury derivative of saligenin



as well as the acetate of this compound and also



and its acetate. The sodium salts of these compounds are water soluble. They have about the same antiseptic action as HgCl_2 and about the same general toxicity but are much less irritant locally to the mucous membranes.

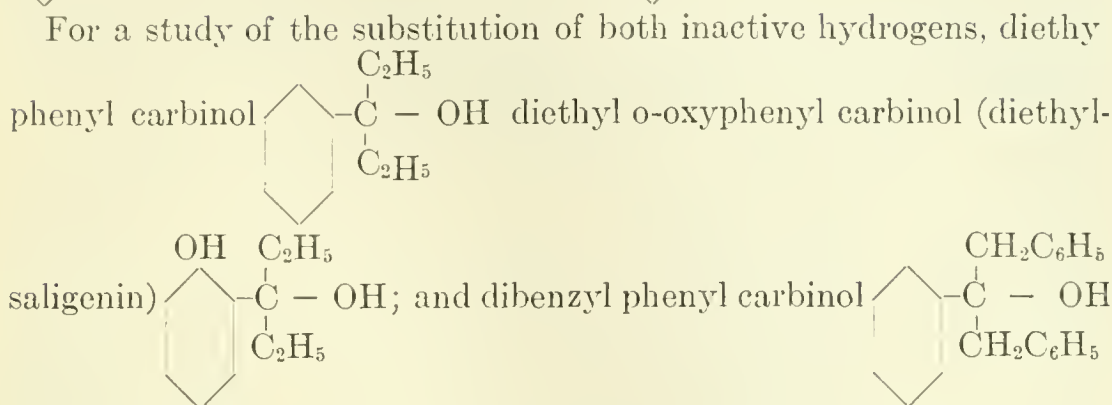
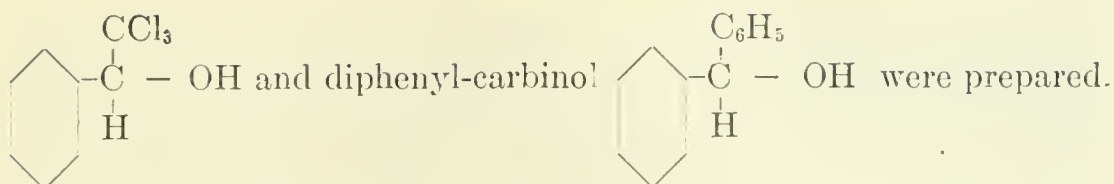
A 1:1000 solution of the sodium salt of mercury saligenin can be held in the urethra for five minutes without causing burning or subsequent irritation. It is therefore being used for the treatment of anterior gonorrhoeal urethritis in the night venereal clinic of the University of Minnesota and is yielding promising results.

None of the substances studied, saligenin, piperonyl alcohol, mercury saligenin and the acetate of mercury saligenin, when injected subcutaneously in rats which had been infected with *trypanosoma brucei* or *spirillum obermeyer*i gave any sign of chemotherapeutic action.

The Relation of Substitution in the Carbinol Group to the Pharmacological Action of Some Phenyl Carbinols. J. PAUL QUIGLEY (by invitation), AND ARTHUR D. HIRSCHFELDER. From the Department of Pharmacology, University of Minnesota.

Macht has shown that benzyl alcohol possesses local anesthetic actions, and Hirschfelder, Lundholm and Norrgaard have shown that the same is true in a greater degree for saligenin, and homosaligenin, but to a somewhat lesser extent for a number of other phenyl carbinols. Hjort and his collaborators have obtained similar results with some related compounds. Moreover, Macht has demonstrated that a substitution for the hydroxyl hydrogen as in benzyl acetate and benzyl benzoate brought about the loss of the local anesthetic action but introduced an antispasmodic action instead.

We have therefore investigated the effects of substitution of one or both of the "inactive hydrogens" in the CH_2OH group. In order to study the substitution of one hydrogen trichlormethyl phenyl carbinol



were prepared.

These are all practically insoluble in water but soluble in alcohol, ether and olive oil. A convenient emulsion can be prepared by dissolving the carbinol in olive oil and emulsifying with acacia in 0.9 per cent NaCl.

Of these compounds the mono substitution products, trichlormethyl phenyl carbinol and diphenyl carbinol when applied in the form of the emulsion to the frog's sciatic nerve, produced sensory block, but no motor block. The sensory block disappeared as soon as the drug was removed. The diethyl phenyl carbinol and dibenzyl phenyl carbinol had no effect; and the diethyl o-oxyphenyl carbinol also produced a slight sensory block. In the latter case the sensory block was probably due to the presence of the phenolic hydroxyl group, because the diethyl phenyl carbinol did not produce sensory block.

Subcutaneously in the human forearm 2 per cent emulsion of the diphenyl carbinol and a 7.5 per cent oil solution of dibenzyl carbinol gave no anesthesia, the former gave rise to an ulceration.

Trichlormethyl phenyl carbinol and diethyl o-oxyphenyl carbinol gave rise to a sharp burning sensation on the tongue; diphenyl carbinol gave this to a lesser degree; dibenzyl phenyl carbinol gave none.

All these facts indicate that in the substances studied, substitution of one of the inactive hydrogens of the CH_2OH group lessens the local anesthetic action, and it is further diminished or completely destroyed by the substitution of both inactive hydrogens.

The Pharmacology of Some Amines. P. J. HANZLIK. From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland, Ohio.

The following compounds which are of considerable importance in the arts and to some extent in industry were studied; metaphenylenediamine, paraphenylenediamine, the dimethylparaphenylenediamine and diethylparaphenylenediamine and the normal mono-, di- and

tributylamines. The phenylenediamines were found to be more toxic than the butylamines and of the phenylenediamines the dimethyl is about twice as toxic as the diethyl derivative, and both derivatives are more toxic than their parent substance, paraphenylenediamine, and the metaphenylenediamine. The toxicity of the butylamines increases with increase in molecular weight, the most toxic compound being the tributylamine. A brief summary of the various actions of the amines studied is presented in the accompanying table.

COMPOUND	APPROXIMATE ORDER OF TOXICITY (1 = MOST TOXIC)	VAPOR TOXICITY	SKIN IRRITATION AND VESICATION	CIRCULATION	RESPIRATION	TEMPERATURE	SMOOTH MUSCLE OF EXCISED ORGANS	CONVULSANT ACTION	PRODUCTION OF EDEMA AND EXUDATES
<i>Phenylenediamines</i>									
Dimethylparaphenylenediamine	1	+	+	Stimulates*	Stimulates	Lowers	Markedly augments tonus	+	-
Diethylparaphenylenediamine	2	+	+	Stimulates*	Stimulates	Lowers	Augments tone	+	-
Paraphenylenediamine	3	-	-	Mild stimulation	Stimulates	Lowers	Increases amplitude of contraction	+	+
Metaphenylenediamine	4	-	-	Mild stimulation	Stimulates	Lowers	Increases amplitude of contraction	+	+
<i>Normal butylamines</i>									
Tributylamine.....	1	-	-	Depresses†	Stimulates	Lowers	Augments peristalsis and tonus	+	-
Dibutylamine.....	2	+	-	Depresses†	Stimulates	Lowers	Augments peristalsis and tonus	+	-
Monobutylamine....	3	+	-	Depresses†	Stimulates	Lowers	Augments peristalsis and tonus	+	-

* Increased pulse rate, blood pressure, kidney volume and cardiac systole.
† Reduced pulse rate, blood pressure, kidney volume and increased cardiac volume.

The Effect of Hemorrhage on the Sympathetics. HUGH McGUIGAN, University of Illinois.
Hemorrhage increases the sensitivity of the sympathetics as judged by the response in blood pressure to the injection of epinephrine. This could be either central or peripheral or both. The action seems mainly

peripheral since stimulation of the centres by such drugs as strychnine does not show any increase in the blood pressure response to epinephrine. Also; in favor of a peripheral action is the fact that a purely central modification of the action of epinephrine is very hard to demonstrate, while the effect of hemorrhage is easily shown.

Liberation of Free Salicylic Acid from Salicylate in the Circulation.

P. J. HANZLIK. From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland, Ohio.

The claim that the common absence of a right-sided or auriculo-ventricular endocarditis in acute rheumatic fever during salicylate therapy is due to the liberation of free salicylic acid by virtue of higher CO₂ content of the blood, resulting in local antiseptic qualities, was put to a test on animals (dogs and cats), subjected to the most favorable conditions for the liberation of the free acid. Maximal doses of sodium salicylate were used intravenously. The bloods from the femoral artery and right ventricle of several animals before and during asphyxia (at exitus) were shaken, immediately after withdrawal into neutral oxalate, with various immiscible solvents (ether, chloroform and petroleum ether). The results were entirely negative.

The fatal asphyxias increased the pH of the blood from 7.4 to 6.8. The lowest acidity at which free salicylic acid was demonstrable in "buffer solutions" containing sodium salicylate was from pH 6.8 to 6.5. Since asphyxial blood at death, though only very slightly acid contained no demonstrable free salicylic acid, it is highly improbable that alkaline venous blood of the right heart in rheumatic fever during life contains any. The explanation of the more common occurrence of the left sided (mitral) than right sided endocarditis in rheumatic fever must be sought elsewhere.

Changes in the Blood of a Dog During Heavy Meat Feeding. H. V.

ATKINSON (by invitation). From the Department of Pharmacology and Therapeutics, University of Illinois, College of Medicine.

A dog weighing 9 kilos was stuffed for 7 days with lean beef heart (700 to 900 grams daily) under conditions previously found to produce fat from protein (cf. Proc. Am. Soc. Biol. Chem., 1919, XIII-13) and an increase of 0.05 per cent in the blood fat was detected on the last day.

On the Therapeutic Efficiency of Silver Arsphenamine Sodium. HELEN

DYER (by invitation) AND CARL VOEGTLIN.

Silver arsphenamine sodium containing on an average 20 per cent arsenic and 15 per cent of silver, has recently been introduced as a substitute for arsphenamine. Clinical tests seem to indicate that silver arsphenamine is a more efficient drug for the treatment of syphilis than either arsphenamine or neoarsphenamine, as smaller doses will cause a disappearance of the lesions. The parasitocidal action of silver arsphenamine sodium, obtained from various manufacturers, was

determined in albino rats infected with trypanosoma equiperdum by means of a method recently described by C. Voegtlin and H. W. Smith. It was found that the minimal effective dose of silver arsphenamine sodium on the basis of its arsenic content is approximately 300 per cent smaller than that of arsphenamine or neoarsphenamine. The maximum tolerated dose of the preparation as determined by the official method in albino rats is 140 mgm. per kilo, indicating that the toxicity is slightly greater than that of neoarsphenamine (180 mgm. per kilo).

The therapeutic ratio $\frac{M. E. D.}{M. L. D.}$ is therefore more favorable in the case of silver arsphenamine sodium than in the case of arsphenamine or neoarsphenamine. It remains to be seen however, whether silver arsphenamine is more efficient in bringing about a permanent cure of syphilis.

On the Comparative Toxicity of Alcohol, Caffeine and Nicotine. D. I. MACHT AND WM. BLOOM (by invitation). From the Pharmacological Laboratory, Johns Hopkins University. Read by title.

Two series of investigations were conducted on the subject. In the first investigation the effects of ethyl alcohol, nicotine tartrate and caffeine were studied on the behavior of albino rats in the circular maze. Eighteen animals were used in this research. After the rats had been previously trained to solve the maze problem by finding their way to the center of the maze without any errors, in the shortest period of time, they were injected subcutaneously or intraperitoneally with solutions of the above drugs and the effect of the drugs on the behavior was noted. It was found that the smallest dose of caffeine required to produce a depression, that is to impair the efficiency of the rats' behavior in the maze, was 10 mgm. The smallest dose of nicotine tartrate which produced depression was 0.02 mgm. for the rat of an average medium weight (150 grams). This is equivalent to about 0.00666+ mgm. of nicotine itself. The smallest dose of ethyl alcohol required to produce even a slight impairment in the behavior of the rats was between 40 and 50 mgm.

In the second investigation the effect of caffeine, nicotine and ethyl alcohol was studied on the development and growth of frog larvae. Tadpoles of the same age were placed in solutions of the drugs in various concentrations and the effect of the poisons was studied. It was found that the toxicity of the drugs varied with the age of the larvae, very young tadpoles succumbing much earlier than older ones to the effect of the poisons. In the experiments, however, in which the three drugs were tried on tadpoles of exactly the same species and age it was found that a concentration of ethyl alcohol, 1:100, was less toxic than a solution of nicotine, 1:50,000 and that a solution of nicotine, 1:50,000, was less toxic than a solution of caffeine, 1:10,000.

A Pharmacodynamic Analysis of Cocaine Action on the Cerebrum.

D. I. MACHT AND WM. BLOOM (by invitation). From the Pharmacological Laboratory, Johns Hopkins University. Read by title.

The effect of cocaine and its chemical components was studied on the intelligent behavior of albino rats in the circular maze. Twenty-five rats were used altogether in the investigation. The animals were trained in the circular maze until they were able to find their way from the entrance to the center without making any errors and in the shortest period of time. The drugs studied were then injected subcutaneously and the effect of the same studied after absorption. It was found that 1 mgm. of cocaine produced a marked depression on the behavior of the rats as indicated by incoördination, slowness of movement and loss of memory and intelligence. Smaller doses ($\frac{1}{30}$ to $\frac{1}{10}$ mgm.) also produced distinct depression as indicated by the time of performance and the number of errors made.

An effort was made to ascertain whether cocaine produces a primary stimulation of the cerebrum. For this purpose very small doses of cocaine were injected. It was found that minute quantities of the drug either failed to produce any effect or produced depression and in no case was there a primary stimulation noted.

Injection of ecgonine-hydrochloride and benzoyl-ecgonine produced no effect on the behavior of the rats even when administered in doses much larger than that of cocaine. Injections of sodium benzoate solution produced no effects. Neither was there any depressant or stimulating effect noted after injections of small doses of methyl alcohol solution (1 per cent).

Various mixtures of ecgonine hydrochloride, benzoyl-ecgonine, sodium benzoate and methyl alcohol in different proportions were found to produce very little effect on the behavior of rats. As a result of the various experiments it is therefore concluded firstly, that cocaine as such exerts a depressant action on the intelligent behavior of albino rats, secondly, that in no case even after minute doses of the alkaloid was there a primary cerebral stimulation noted and thirdly, that the various components into which the cocaine molecule can be split up, when injected individually or as simple mixtures of each other, do not cause the same action as their chemical combination in the form of cocaine. The complete data of this investigation will appear in the *Archives Internationales de Pharmacodynamie et de Therapie*.

The Influence of the Oil of Chenopodium on the Heart of the Turtle.

W. SALANT AND BATTLE (by invitation). From the Department of Physiology and Pharmacology, Medical Department, University of Georgia. Read by title.

Oil of chenopodium applied to the heart of the turtle in situ produced marked slowing, the frequency being decreased in some experiments about 50 per cent. There was also diminution of amplitude but this was very gradual. Stimulation of the vagi after treatment with chenopodium was ineffective. This was observed, in some experiments

soon after the application of the oil of chenopodium, in others the inhibitory effect of the oil developed after an interval of five or six minutes. The effect of the oil of chenopodium on the vagi may be removed, however, by pilocarpine as stimulation of the vagi become effective again when a solution of the alkaloid was applied after previous treatment with the oil. When pilocarpine was applied, in the same way as the oil of chenopodium, and the vagi stimulated by the interrupted current, the usual inhibitory effect was obtained. The antagonistic action of the two drugs could also be shown when treatment with the oil of chenopodium followed the application of pilocarpine to the heart. Cardiac depression with oil of chenopodium was also obtained in the atropinized heart.

Further Observations on the Action of Heavy Metals. W. SALANT AND N. KLEITMAN (by invitation). From the Department of Physiology and Pharmacology, Medical Department, University of Georgia. Read by title.

The isolated heart of the turtle and frog was perfused with salts of mercury and lead. Mercuric chloride in different concentrations produced in the turtle's heart marked depression and irregularity of action. Delirium cordis such as observed in higher animals was a frequent occurrence and was obtained with as low a concentration as one part of mercury in ten million parts of Ringer solution. Disturbance of rhythm and heart block were also observed. Tests made with benzoate, acetate and succinate of mercury showed that these salts were less toxic than the chloride. Irregularity of action, including delirium cordis, were absent when the turtle's heart was perfused with the organic preparations.

Observations on the frog's heart indicated that the different mercury compounds were distinctly less toxic than in the heart of the turtle. No delirium cordis appeared; other irregularities were observed, however.

In experiments with lead the acetate was the only salt employed. Pronounced depression was likewise observed in these experiments, but the effect was less marked than in the case of mercury. Even in experiments in which toxicity was very marked, improvement, and sometimes recovery, occurred when perfusion with lead was discontinued, and Ringer solution substituted.

A Direct Method for Carbon Monoxide in Blood. THEO. K. KRUSE.

The method which was described is a modification of the Van Slyke-Salvesen method.¹ It differs from it in that the carbon monoxide is determined directly by absorption with ammoniacal cuprous chloride instead of subtracting a constant volume of nitrogen from the residual gas.

The principle of the method is to liberate oxygen and carbon monoxide from hemoglobin by treatment with potassium ferrieyanide and to

¹ Van Slyke and Salvesen: Jour. Biol. Chem., 1919, xl, 103.

collect these gases by evacuation in the Van Slyke blood gas apparatus. Strong alkaline pyrogallate is added to absorb the oxygen, and ammoniacal cuprous chloride to absorb the carbon monoxide. The tension of ammonia vapor has been so small that addition of water for its absorption has not altered the final reading.

Clinical and Experimental Studies in Diabetes insipidus. E. E. LARSON (by invitation), J. F. WEIR (by invitation), AND L. G. ROWNTREE. From the Mayo Clinic, Rochester, Minn.

Clinical results in fourteen cases of diabetes insipidus treated with (commercial) extracts of posterior lobe and pars intermedia. Effects of pituitrin and histamine contrasted. Metabolic studies in diabetes insipidus during pituitrin treatment, show decreased urinary excretion of water intake, acid bodies, and, to a less extent, of nitrogenous bodies. The blood shows water retention, decreased chloride content, and frequently decreased molecular concentration. Forcing water in diabetes insipidus during oliguria resulting from pituitrin results in increased blood volume and water intoxication, and, in one instance, in demonstrable oedema. Pituitrin raises the water threshold of the kidney in diabetes insipidus.

Effect of Tobacco Smoking on Human Sensory Thresholds. WALTER L. MENDENHALL. From the Department of Pharmacology, Dartmouth Medical School.

A series of observations were made on trained subjects, both smokers and non-smokers, whose thresholds were determined by the Martin method of quantitative faradic stimulation. The index and middle fingers of the right hand were immersed in salt solution electrodes; the subject sat in a reclining chair, free from disturbance of any sort. By means of an electric key, he could signal the operator whenever the threshold had been reached. β units were estimated in all instances. The usual routine was: the subject's normal threshold was first determined and then after smoking had been indulged in for twenty to thirty minutes, a second threshold was measured.

The observations demonstrated that the effect of smoking as shown by variation in the threshold, was conditioned upon the state of the sensory mechanism at the time of observation. The average normal threshold of both smokers and non-smokers was about 180 β units. If the subject's threshold was at or near normal, smoking was not usually very effective in changing it, whereas if his threshold was low indicating high irritability (nervousness), then smoking depressed the irritability quite markedly, in some instances as much as 400 per cent; if the subject's sensory mechanism was in a depressed state (high threshold), then smoking had a stimulating effect (lowered the threshold). The depressant effect of smoking was much more marked than was the stimulating effect.

Control experiments were made in which the subject rested instead of smoked. The effect of resting upon the sensory threshold was to

vary it in the same direction as did smoking. Thus, if the subject was nervous or irritable (low threshold), resting tended to raise the threshold and bring it back toward normal; on the other hand, if the subject was depressed (high threshold), then rest tended to lower the threshold toward normal.

The effect of smoking cubeb cigarettes was tried. The usual result was a stimulation of the sensory mechanism, no matter what the normal threshold was. In other words the effect of cubebs did not seem to be conditioned upon the state of the sensory mechanism at the time of the observation.

Just as rest tended to bring the sensory threshold back toward normal, so did smoking tend to bring it back toward normal, but smoking differed from rest in that it was much more effective in causing a return to normal than was rest.

These results may offer a possible explanation for the widespread use of tobacco. Since feeling or sensation is an expression of our physical state, it would be logical to conclude that man feels best when he is in a normal condition. Rest has the effect of causing a return towards normal; according to these observations smoking has a like effect only more marked. The stimulating action of smoking when one is depressed and the depressing effect when one feels nervous or irritable fits in with the above observations in which the threshold was accurately measured. Non-smokers showed the same effects as did smokers. No explanation is offered as to how the effects are exerted, since no experiments were done in which the relative influence of rest, carbon monoxide nicotine and psychical factors on the threshold were evaluated.

Some Factors in the Production of Acid Fuchsin Convulsions in Frogs.

J. E. THOMAS, West Virginia University.

With the view (in the beginning) of obtaining additional evidence of the inhibitory influence of the cerebral hemispheres on spinal convulsions, the effect of the following procedures was tried on frogs poisoned with acid fuchsin: (1) Removal of one cerebral hemisphere only. (2) Injuries to or removal of the olfactory lobes. (3) Injuries to the brain too slight to interfere seriously with function. (4) Section of or injuries to the spinal cord at different levels.

All these procedures were found to hasten the onset of convulsions, in most cases fully as effectively as removal of the anterior one-third of both cerebral hemispheres. They served to bring on convulsions promptly in frogs that had received from one-tenth to one-fourth the minimum convulsant dose for unoperated frogs. These results were obtained whether the operation preceded or followed injection of the drug.

In the case of destruction of one cerebral hemisphere only, the convulsions showed the usual bilateral symmetry, and did not involve one-half of the body more than the other as would be expected if the onset of convulsions were determined by the release of spinal centers from cerebral inhibition. After injuries to the olfactory lobes convul-

sions appear after a somewhat longer time than was allowed by Barbour and Abel in similar experiments. Of the slight injuries to the brain, a simple pin prick in the region of the optic lobes, or just anterior to them, is as effective as decerebration in bringing on convulsions. In the case of injuries to the spinal cord the rapidity of the onset of convulsions was found (with constant dosage) to vary with the distance of the injury from important motor centers. In many cases local tetanus was observed at the site of the injury. If the cord was sectioned convulsions arose in both ends, and frequently earlier in the anterior end than in the posterior end, the determining factor being the nearness of the section to the medulla and associated motor centers. This fact is regarded as significant because in the experiments as performed there could have been no injury to the cerebral hemispheres.

From these observations the conclusion is drawn that cerebral inhibition is a minor factor, if it is a factor at all, in determining the time of onset of acid fuchsin convulsions in frogs.

The work was undertaken at the suggestion of Dr. Joseph. Part of the experiments were done at the Iowa Lakeside Laboratory with apparatus furnished by St. Louis University, and part in the Physiology Laboratory of West Virginia University School of Medicine.

A Study of Thyroid-Iodine Distribution and Mobilization. H. B. VAN DYKE (by invitation). From the Laboratory of Physiological Chemistry and Pharmacology, University of Chicago, Chicago.

The distribution of iodine between cells and colloid was determined in the thyroid glands of man, of normal dogs, and of iodine-fed dogs.¹

Rahe et al. and Watts have maintained that a three to four hours' stimulation of the cervical sympathetic nerve in the dog causes a diminution of the iodine content of the lobe of the thyroid gland on the side of the stimulation. In connection with some studies of the distribution of iodine in the dog's thyroid gland, their work, with some modifications of the technique of iodine determination of the glands used, was repeated but was not confirmed.

Experiments on the Variability in Susceptibility to Poison Ivy. E. D. BROWN. From the Department of Pharmacology, University of Minnesota.

Experiments were performed on groups of students to determine whether they were susceptible to poison ivy.

In one group of nine members a portion of the fresh leaf about 1 cm. square was applied to the arm, covered with a strip of adhesive plaster and allowed to remain overnight. On six members of the group there was a reaction after a period of twenty-four hours to six days after the drug had been applied. On three members of the group there was no reaction.

¹ To be published in full in the Journal of Biological Chemistry for January, 1921.

On a second group of nine members a drop of tincture prepared from the fresh leaves was applied. A reaction occurred on seven members of the group in two to three days, while on two members there was no reaction. One of the members of this group was not affected by the leaf, but responded to the tincture.

A substance was extracted from the leaves having the appearance and properties of a fixed oil, (probably the toxicodendrol isolated by Pfaff), was applied to a large number of individuals and in no case did it fail to produce a reaction. The degree of reaction from the plant or from a preparation of the plant was found to vary from a pruritus without visible evidence of irritation, to a raw surface. This variability in reaction depends also upon the susceptibility of the individual. There may be an erythema with slight induration, a macular eruption, a vesicular eruption and eventually a sloughing of the cutaneous tissue.

This reaction is apparently specific for the skin, for in no instance has there been any involvement of the mucous membranes even when the leaves have been chewed.

The serum exudate from the vesicles applied to other parts of the body, and to scarified areas, in no instance gave any reaction.

The poison applied to the skin and carefully protected so that it could not be spread to other parts of the body through the agencies of the hands or clothing, was not effective against spreading of the eruption.

It must therefore be carried to surrounding areas by means of either the lymphatics or blood vessels.

It was found that with those who had been once poisoned by the plant their susceptibility to the poison was increased.

Studies on the Cardio-Inhibitor Center. F. B. BECHT, Northwestern University Medical School.

The effect on the heart of stimulation of the central end of the left vagus with the right intact was studied on dogs under ether, morphine, paraldehyde, urethane, chloral, chloretone, and under normal conditions. It was found that under ether, reflex inhibition was obtained in 40 per cent; acceleration in 33 per cent; and no change in 26 per cent of 95 observations. Under the influence of all the other drugs enumerated and under normal conditions inhibition was noted in 100 per cent in 53 cases. Complete reflex inhibition was noted in 2 of 8 cases under morphine, in 1 of 7 cases under paraldehyde, and in 2 of 6 cases under urethane. Since complete reflex cardiac inhibition was never noted under normal conditions it was concluded that the cardio-inhibitor center is depressed under ether, stimulated under morphine, paraldehyde and urethane, and practically normal under chloral and chloretone.

Epinephrine Hyperglycemia. ARTHUR L. TATUM. From the Laboratory of Pharmacology of the University of Chicago, Chicago.

This work embodies chiefly an analysis of the factors of interrelationship between epinephrine induced glycogenolysis and the subse-

quent status of alkaline reserve capacity of blood as suggested by the work of Ritzmann and of Peters and Geylin.

1. Phloridzin sufficient to produce hypoglycemia produces a fall in alkaline reserve capacity of whole blood.

2. Epinephrine injected subcutaneously in conditions of low reserve from "phloridzin acidosis" causes a rise in blood sugar without further fall in alkaline reserve capacity of blood.

3. Hydrochloric acid by stomach produces a marked fall in reserve capacity with but little change in sugar. Epinephrine injected under these conditions produces the usual amount of glycogenolysis with no further significant change in alkaline reserve capacity.

4. For a given fall in reserve by hydrochloric acid by stomach and by epinephrine subcutaneously injected, the resultant glycogenolysis from epinephrine is so very much greater than that produced by hydrochloric acid as to be essentially incomparable.

5. Previous induction of acidosis by hydrochloric acid does not considerably increase or decrease the efficacy of epinephrine as a glycogenolytic agent.

6. All in all, there appears to be no demonstrable evidence whatsoever of any interdependence between epinephrine glycogenolysis, as judged by hyperglycemia, and the fall of alkaline reserve capacity as usually found to occur after subcutaneous injections of epinephrine in normal animals. Glycogenolysis and acid production appear to be merely concomitant and independent phenomena following epinephrine injections.

On Decreasing the Reaction of Normal Skin to Destructive Doses of X-rays by Pharmacological Means, and on the Mechanism Involved. JOHN AUER AND W. D. WITHERBEE (by invitation). From the Laboratories of the Rockefeller Institute.

Ten rabbits were sensitized by four, spaced, subcutaneous and inter-muscular injections, each of 1 cc. horse-serum. Ten days after the last sensitizing dose these rabbits and five additional normal ones were rayed locally with thirty skin units (Remer-Witherbee formula): Coolidge tube, 3-inch spark gap, 10 milliamperes, 6-inch distance from target, twenty-minute exposure. The site rayed was always an area of 4 sq. cm. in the upper half of the right ear; the rest of the body was protected by a sheathing of lead. It should be observed that this arrangement subjects two skin surfaces to the action of the rays; one surface, the dorsal, was affected by the rays on entry, while the internal skin surface was acted upon by the rays on their exit.

Thirteen days after the raying and twenty-three days after the last sensitizing dose of serum, five rabbits of the sensitized group and the five normal controls were injected intraperitoneally with 10 cc. of horse-serum. We have thus three groups of animals, all of which had been rayed in exactly the same way: five were normal rabbits injected once with horse-serum, thirteen days after raying (horse-serum controls); five were sensitized but *not* reinjected; they had been rayed ten days after the last sensitizing dose (sensitized group); and five were sensi-

tized and rayed like the preceding group but were reinjected with serum thirteen days after raying (sensitized-reinjected group). These groups were now carefully observed for months; each group lost one member by death, the final groups therefore consisted of four rabbits each.

The results are briefly as follows.

The *horse-serum controls* showed dry gangrene and fenestration in the rayed area of the ears in 36, 47, 50, 50 days respectively.

The *sensitized-reinjected group* developed dry gangrene and fenestration in 50, 52, 62, 85 days respectively.

The *sensitized group*, however, responded quite differently on the whole. One rabbit developed gangrene and fenestration in 46 days. A second rabbit showed the same result but only after the lapse of 131 days. The two remaining rabbits show permanent alopecia, smooth, thin, skin surfaces, and no sign of inflammation at the present time, over 220 days after raying.

It is possible that this increased resistance due to sensitization may be of some practical use to the human subject when x-rays must be used in heavy doses. For example, in more or less deeply seated cancers the dose of rays applicable is definitely limited by the tolerance of the overlying skin, and a lethal dose for the malignant cells frequently cannot be given because the skin cells would be destroyed. This therapeutic possibility, however, presupposes that the sensitization does not increase the resistance of the cancer cells to the same degree as that of the skin cells.

A number of inferences of considerable theoretical interest may be derived from the experimental data described above, if we accept the general view that an anaphylactic reaction is initiated by the union of antigen and anaphylactic antibody.

The local protection to x-rays seen in the *sensitized group* may be explained by the presence of anaphylactic antibodies in this group of animals.

The lack of protection seen in the *sensitized-reinjected group* may be considered due to the relative absence of these reaction-bodies, for it is a generally accepted experimental fact that the anaphylactic antibodies disappear during an anaphylactic reaction, and such a reaction did take place in the sensitized-reinjected group. This anaphylactic reaction is the only factor which differentiates the *sensitized-reinjected group* from the *sensitized group*.

Another inference is that the protection which the anaphylactic antibodies give, must be due to those reaction-bodies which are anchored to the tissue cells, and not to those which are circulating. This is shown by the *serum control group*. In these animals the antigen was injected intraperitoneally thirteen days after the local raying of the ear. Within relatively few days an abundance of anaphylactic antibodies must have been circulating and must have passed through the capillaries of the rayed area, yet no protection was afforded for gangrene in the rayed area occurred within 50 days after raying.

Finally it follows directly that a general anaphylactic reaction can dislodge those anaphylactic antibodies which are anchored to the tissue cells and thus remove the protection which they give to x-rays. This is shown by *sensitized-reinjected group*, where a reinjection of antigen largely abolished the protection which mere sensitization previous to x-ray gives, and which is illustrated in the *sensitized group*.

The Action of Magnesium Salts Upon the Mammalian Heart. S. A. MATTHEWS, Loyola University School of Medicine, Chicago.

1. Magnesium sulphate. A general protoplasm depressant, the depressant action taking the form of a gradient.

2. A certain size dose will bring the heart to a standstill similar to the inhibition caused by vagus stimulation, and like vagus stimulation the standstill is of short duration; that is the contraction waves will break through.

3. After the minimal dose of magnesium sulphate to stop the heart, stimulation of the accelerator nerves will restore the beat, and after a larger dose stimulation of the sino-auricular node will restore the beat.

4. Magnesium salts depress the conduction mechanism of the heart and when the mechanism is depressed to a certain degree, all conduction stops and the heart comes to a standstill; but when a stronger stimulus is brought into play by stimulating, electrically, the accelerator nerves or better the auricle, conduction may be brought back, although a degree of depression may be reached where no degree of stimulation will be effective.

5. After complete depression of the accelerator nerves and of the conduction apparatus, the heart muscle will respond to direct stimulation, and when applied to the right ventricle, conduction will pass to the left ventricle so that an efficient beat may be maintained by direct right ventricular stimulation.

6. After the quiescent heart has been stimulated directly (right ventricle) at a rate of one hundred stimuli per minute for 10-12 minutes it will start beating of its own accord.

7. After the accelerator nerves have been stimulated it takes a much larger dose of magnesium sulphate to stop the heart than before such stimulation.

8. The heartbeat may be slowed to a degree that the course of the impulse over the heart is made visible, and seems to correspond with the normal electrocardiogram of the heart.

9. As suggested in another paper, already published, it seems that it is possible to depress the nervous and conduction mechanism of the heart to a degree where all spontaneous beats cease before any marked depression of the heart muscle becomes apparent. If so, it may serve as a means of separating the nervous mechanism of the heart from the musculature, and be a means of proving whether or not the normal heartbeat in mammals is dependent upon the degree of irritability of the intrinsic nerves of the heart.

The Pharmacological Action of Organic Lead Compounds. E. C. MASON (by invitation). From the Department of Pharmacology of the University of Cincinnati, College of Medicine.

Work on this subject has been confined mainly to compounds of triethyl lead. Triethyl lead is a heavy oil with a specific gravity of 1.471 at 10 degrees centigrade and corresponds to the formula of $(C_2H_5)_3Pb$. It is rather insoluble in water and for that reason I have used mainly the chloride and the acetate. They have the formula of $(C_2H_5)_3Pb\ Cl$ and $(C_2H_5)_3Pb\ (C_2H_3O_2)$ respectively.

In all of these preparations the lead is in the masked form which means that reagents such as chlorides, sulphates and sulphides do not precipitate the lead in this combination. Also lead in this form when injected into the blood stream, does not precipitate the blood proteins. It is therefore possible to inject solutions of these compounds intravenously, which I did in 0.5 per cent water solutions.

These compounds were first prepared by Lowig (1853) and Klipple (1860) and again later (1878) by Harnack who tested their action on animals. However, the work which he did at that time was necessarily limited.

The injection of 0.5 cc. of 0.5 per cent solution (0.0025 grams) intravenously, into a dog of 5 to 10 kilos produces marked changes in respiration, blood pressure, spleen volume kidney volume and intestinal activity. The *first injection* has given the constant result of extreme fall in blood pressure, great respiratory disturbance, either a dilatation of the kidney and spleen or an initial dilatation followed immediately by a constriction and an increased intestinal activity. The *second injection* of the same size dose gives an entirely different picture. The blood pressure, instead of falling rises quite similarly to the result obtained from an adrenalin injection. With suitable doses in many instances when compared with adrenalin the rise is greater and lasts much longer. The respiration is greatly stimulated, kidney volume and spleen volume decrease, and in general the picture is quite the reverse of that produced by the first injection. Subsequent injections give results similar to those obtained from the second injection; however, often much more marked.

In attempting to determine the point of action of these compounds, it appears that there is some action on the vagus system. For after repeated injections, and often after one injection, it is found that stimulation of the vagus in the neck has no effect on the heart. However, the characteristic action on blood pressure and respiration is produced after atropine and after both vagi are cut.

The compounds are peculiar in their power of stimulation. They are so active in this capacity that special care is used to have the animals deeply under ether at the time of administration; otherwise they appear almost to regain consciousness. These preparations, when administered to pithed animals, show no action on blood pressure.

The Relation Between the Blood Coagulating and the Smooth Muscle Contracting Properties of Tissue Extracts. C. A. MILLS (by invitation). GERARD RAAP (by invitation) AND D. E. JACKSON. From the Departments of Pharmacology and Biochemistry, University of Cincinnati, College of Medicine.

The experiments herein reported have dealt with the pharmacological and physiological properties of extracts made from various tissues, but more especially those made from lung tissue. Six different extracts or fractions thereof have been used. (1) The first of these which we have designated as "lung extract" has been made by grinding up 10 per cent of dried beef lung with 0.9 per cent NaCl solution. This mixture was centrifuged and the clear portion decanted off from the precipitate. It contains 0.74 per cent protein and gives a positive imidazol test (Ehrlich). (2) The second solution we have called "crude antithrombin." It is made by extracting dried beef lung with benzine at room temperature and then grinding the extracted lung material up in 0.9 per cent NaCl solution. This extract contains 0.93 per cent of protein and gives a positive Ehrlich reaction. (It contains antithrombin globulin and albumin). (3) The next solution we call "albumins of lung extract." This is made from (1) by removing the globulins by acid precipitation ($N/500\text{ H}_2\text{SO}_4$). It contains 0.16 per cent proteins and gives a positive imidazol ring test. (4) "Purified antithrombin" is the antithrombin globulin of (2) precipitated by acid ($N/500\text{ H}_2\text{SO}_4$) from the "crude antithrombin." The precipitate is washed and redissolved in 0.9 per cent NaCl solution by the addition of a small amount of alkali. It contains 0.43 per cent of protein and does not give a positive imidazol test. (5) "Purified coagulant" is made by precipitating the active globulin from "lung extract" (1) by acid ($N/500\text{ H}_2\text{SO}_4$), washing the precipitate and redissolving it in 0.9 per cent NaCl solution by the addition of a small amount of alkali. It contains 0.23 per cent of protein and is free from histamine (negative imidazol test). This solution contains active globulin only. (6) "Purified coagulant (inactivated)" is made from "purified coagulant" (5) by adding enough $N/2\text{ NaOH}$ solution to give an approximate alkalinity of $N/15$, boiling and then bringing the mixture back to within a few drops of neutrality by the addition of $N/2\text{ H}_2\text{SO}_4$. This extract retains only a small fraction of its original coagulating activity. It contains 0.2 per cent protein and is histamine free (negative imidazol test).

The chief physiological property of "active coagulant" (5) is the power it possesses to rapidly cause intravascular clotting of the blood when a small amount is injected intravenously. The "inactivated coagulant" (6) fails to thus cause clotting.

If a small dose of "lung extract" (1) is injected intravenously into a pithed dog, the blood rapidly clots in the vessels, but at the same time there is produced a profound contraction of the bronchioles, bladder and uterus. We have studied only these organs so far, but we presume that others are also involved. A large dose of "crude anti-

thrombin" (2) contracts the bronchioles, but does not clot the blood. "Albumins of lung extract" (Ehrlich positive) contract the bronchioles, but fail to clot the blood. "Purified antithrombin" (4) does not affect the blood, but may exercise a very slight and much-delayed contracting action on the bronchioles. The contraction, however, does not resemble that produced by histamine in its general appearance. "Purified coagulant," when injected intravenously, causes immediate intravascular clotting and at the same time in pithed dogs (brain and cord) produces profound contraction of the bronchioles, bladder and uterus.

The injected solution here contains no histamine (imidazol ring negative). In contrast to this the "inactivated coagulant" (6) produces no clotting of the blood and no response whatever from the smooth muscle of the bronchioles or bladder. This solution is also, of course, free from histamine. It seems obvious that when the blood clots, the bronchioles, uterus and bladder are affected in such a way that contraction of their muscular fibers is produced. If excised strips of guinea-pig's uterus are suspended in horse plasma (oxalated) and "active coagulant" (5) and finally calcium chloride solution are added to clot the plasma, then just as clotting occurs, the uterus strips contract. Two explanations of these phenomena have been suggested, first, the possibility that the mechanical processes involved in the sudden formation of extensive clots inside the blood vessels may cause the smooth muscle fibers to contract, and second, the view that in some way, just at the moment the blood clots, it or tissues directly associated with it, suddenly liberates from combination with the proteins, some substance resembling histamine, or possibly histamine itself.

A Method for the Study of the Permeability of the Meninges for Arsenicals.

M. I. SMITH AND CARL VOEGTLIN.

The failure to obtain favorable results in many cases of syphilis of the central nervous system by intravenous medication with the newer arsenicals has been attributed to the permeability of the meninges for these specifics. This contention is largely based upon the finding of only small amounts of arsenic in the cerebro-spinal fluid following the intravenous injection of arsphenamine or of neoarsphenamine. The arsenic content of the cerebro-spinal fluid does not however afford positive evidence as to the therapeutic efficacy of a given arsenical, for the arsenic may be in an inactive form, if present, or in a highly active form, though small in amount. A direct biological method has therefore been devised for the study of the permeability of the meninges. This briefly consists of an injection of a heavy suspension of trypanosoma equiperdum into the subarachnoid space of rabbits, and followed by an intravenous injection of the arsenical. After twenty-four hours the cerebro-spinal fluid is examined for trypanosomes, and if none are found it is taken as evidence that the arsenical under examination reached the cerebro-spinal fluid in sufficient amount to be trypanocidal.

Studies by this method indicate that the normal meninges are per-

meable for both arsphenamine and for neoarsphenamine, if administered in adequate doses. A considerable variation in the results was noted in different rabbits injected under uniform conditions with the same drug and with the same dose.

A Preliminary Note on the Colorimetric Estimation of Morphine. H. J. CORPER (by invitation) AND HARRY GAUSS (by invitation). From the Research Department, National Jewish Hospital for Consumptives, Denver, Colorado.

A colorimetric method for the estimation of morphine in amounts of from 0.05 to 50 mgm. by the use of Marquis reagent has been developed. This reagent has been found to have an extinction point at about 0.003 mgm., with approximately the same delicacy as the reaction obtained with Lafons reagent.

An analysis of certain market tablets of morphine sulphate by the colorimetric method revealed a fairly uniform content of the alkaloid. Various methods of extraction of the alkaloid from an aqueous mixture containing organic substances by means of chloroform were studied. To completely extract 5 mgm. of morphine from an aqueous solution alkalized with sodium bicarbonate required 8 extractions with hot chloroform. In the analysis for morphine in varying amounts (from 1 to 32 mgm.) added to agar only the larger amounts were recovered quantitatively.

Clinical Observations on the Absorbability of Purified Tincture of Digitalis.

R. W. SCOTT. From the Department of Medicine, Western Reserve Medical School.

A series of hospital patients was used for this study. The drug administered was a chloroform soluble digitalis body, prepared by Dr. R. A. Hatcher and standardized so that 1 cc. = 1 cat unit. Studies were made of (1) the clinical evidence of absorbability as shown by the rapidity with which therapeutic effects are induced and (2) the promptness with which a change appears in the T-wave of the electrocardiogram after the drug is administered orally.

Clinical studies were made on fourteen adults with auricular fibrillation showing varying degrees of circulatory failure. A single 10 cc. dose of the drug was given and hourly observations were made on the patient's general condition together with careful counts of the apex and radial rates. An initial digitalis effect occurred in from one to two hours with a maximum drop in heart rate in from six to twenty-four hours in nine instances.

To eliminate the effect of stasis on the absorption of the drug thirteen observations were made on ten patients who had no signs of circulatory failure. Except in one instance the dosage used in this series was 0.1 cc. per kilo body weight given in a single dose. Standardized electrocardiograms were taken before the drug was administered and at hourly intervals thereafter for four to five hours. In eight experiments on five individuals a definite effect on the T-wave of the electrocardiogram

occurred in one hour after the drug was administered. In one hour after the administration of 0.15 cc. per kilo, the electrocardiogram in a case of auricular flutter showed definite slowing of the ventricle from increased block and two hours after the drug a record typical of auricular fibrillation was obtained.

No effects were seen in the records of four patients with syphilitic myocarditis even twenty-four hours after receiving the drug.

The conclusions reached from this study are: 1. Absorption of therapeutic doses of this digitalis preparation orally administered occurred in from one to two hours as indicated by the slowing of the heart in patients with auricular fibrillation. Strict uniformity of results were not seen in some individuals with marked circulatory stasis.

2. In patients without stasis absorption occurred in the majority of patients in one hour after doses of 0.1 cc. per kilo body weight were administered. Exceptions to this were individuals with syphilitic myocarditis.

The Toxicity of Strychnine for the Brown Bat (Epstesicus fuscus). ERICH W. SCHWARTZE.

Though there were but three experiments these are of interest because of the paucity of data on the subject in the literature and the necessity of the collection of information of this kind even though it be piecemeal. Kobert and Kunkel state that the lethal dose is 40 mgm. per kilo (species of bat and observer not given). Only three large brown bats (*Epstesicus fuscus*) were available and were used about November 1, 1920. They were rather active and did not appear to be hibernating but were nevertheless warmed up before using. The doses administered were 1.5, 2.5 and 3.5 mgm. strychnine sulphate per kilo. As none survived, it cannot be said what the minimum lethal or the sub-lethal doses might have been. It is certain, however, that 1.5 mgm. per kilo is fatal. The behavior of these bats was interesting in that convulsions were clonic consisting of very rapid movements with only the briefest momentary cessation or period of relaxation. Voluntary jaw movements occurred practically throughout the spasm period. The duration of the experimental periods were 15 to 20 minutes.

The above indicates that these low mammals with a low form of nervous system are a valuable experimental means in the study of the comparative pharmacology and physiology of the nervous system because of the low resistance to this drug, the type of spasm and the possibility of studying the effect of hibernation.

The Relation of Cottonseed Poisoning to Gossypol. CARL L. ALSBERG AND ERICH W. SCHWARTZE.

The relation of the influence of source (climatic and varietal factors) of ground cottonseed meats or kernels to their ability to produce acute death and chronic intoxication has been studied in rats. Four different lots of seed containing different amounts of gossypol were used. These lots were representative of the regional and annual variations occurring

and were selected after making a large series of chemical analyses. Only the edible portion (kernel) was studied. Gossypol has been fed to rats and cats, and to a small extent to mice and rabbits for the purpose of determining the species resistance or threshold of intoxication and the clinical course of the intoxication.

The results indicate: (1) Variation in the gossypol content due to seasonal, regional and climatic conditions was found. A relation is apparent between the gossypol content and the amount of nitrogen and fat the kernels contain. (2) The existence of a close relation between the gossypol content and the ability of this seed to produce acute and chronic intoxication was found. (3) It has been possible by feeding gossypol in an adequate diet to suitable subjects, and by careful attention to the amounts fed in order to avoid inanition to produce the varying phenomena observed in cottonseed poisoning, viz.: (a) Paralysis and paresis of the limbs with myelin degeneration of nerves. (b) Acute attacks of inspiratory *dyspnoea* accompanied by oedema of the lungs. (c) Hydrothorax and ascites with dilation and hypertrophy (increased weight) of the heart. (d) Stunting of growth or loss of weight. (e) Gastro-intestinal phenomena such as loss of appetite, diarrhea, vomiting (in cats) and impaired digestion (decrease in absorption of nitrogen). (4) In addition we should like to report for the first time a single preliminary observation of an increased output of nitrogen in the urine, when gossypol was fed to cats in a meat diet, despite a poorer absorption of the intestine.

One of the fortunate observations of those experiments in which gossypol has been fed is the confirmation as to the character of the intoxication of numerous observations of others who were not in sympathy or whose conclusions were alien to the gossypol hypothesis.

The Determination of the Circulation Time in Dogs and Rabbits and its Relation to the Reaction Time of the Respiratory Center to Sodium Cyanide. E. G. SEYBOLD (by invitation), A. S. LOEVENHART AND B. H. SCHLOMOVITZ.

At the meetings last year, we reported certain improvements in the determination of the circulation time in rabbits. We have since altered the method, substituting lithium benzoate and lithium acetate for the chloride and find that the lithium salts of the organic acids are preferable inasmuch as there is less injury to the vein.

There is no doubt, however, that hexamethylenetetramin is the most inert substance from the physiological standpoint that we have studied which is adapted for the accurate determination of the circulation time.

We have extended the work to dogs, using small cylindrical tubes in place of the cover-slips previously described. The tubes are attached to a piece of rubber adhesive, which is in turn fixed to the kymograph paper. In the same animals we have determined the reaction time to sodium cyanide and indeed often simultaneously with the circulation time. The results are shown in the following table. The number of

experiments used in arriving at these averages is indicated in the numbers in parentheses:

	CIRCULATION TIME			REACTION TIME
	Lithium salt	Hexamethylen-tetramin	Sodium ferrocyanide	
Rabbit.....	4.63 (7)	4.04 (5)	4.47 (8)	{ 3.9 (50) 21 rabbit
Dog.....	7.95 (8)	7.92 (8)		{ 8.68 (75) 17 dogs

Table 2 shows the relation of circulation time to the reaction time of the respiratory center to sodium cyanide on intravenous injection:

		REACTION TIME	CIRCULATION TIME IN TERMS OF REACTION TIME = 100
Rabbit.....	4.38	3.9	115.0
Dog.....	7.94	8.68	91.5

The table shows that in the rabbit the circulation time is 15 per cent slower than the reaction time to the cyanide, while in the dog, the reaction time is 8.5 per cent slower than the circulation time.

In both rabbits and dogs, however, the circulation time and the reaction time differ from one another less than one second.

Hence we may conclude that the reaction time of a given animal to sodium cyanide is approximately equal to the complete circulation time in that animal.

The Toxicological Action of Azulene. JULIA WHELAN (by invitation), C. S. LEONARD (by invitation), AND A. S. LOEVENHART.

The blue hydrocarbon azulene was originally obtained by the distillation of the oil of wormwood. Our material, furnished by the Department of Pharmaceutical Chemistry, was obtained from *Achillea millefolium*. The compound has the composition C₁₅H₁₈ and is as blue as indigo. It is soluble in the organic solvents and insoluble in water. The hydrocarbon is somewhat irritating locally. It has a slight camphor-like odor. On administration by stomach to rabbits, the oil causes a slight diarrhea in a majority of cases and the pigment appears in the feces, probably in altered form. The oil is quickly absorbed from the gastro-intestinal tract and is excreted in the urine as an altered product which is of a greenish color. The urine always contains albumin for some days and the excretion of the pigment also continues for a number of days. The urine finally becomes brown in color before it becomes normal. The most prominent symptoms shown by the animal are: loss of appetite, weakness, tremor and, in some cases, con-

vulsions. In all cases where convulsions were noted, meningeal hemorrhages were observed, between the cerebral and cerebellum and, in all cases, there was congestion of the meningeal vessels. The most characteristic pathological findings, however, are in the kidney. If the animal dies within a relatively short time, the kidney has a dark grayish-green color. The cortex also has this color on section, but the medulla is free of it. Microscopical examination of the kidney shows that the lesion consists of greenish stained necrotic cells in the tubules. The glomeruli are apparently entirely unaffected.

The fatal dose of the drug for rabbits may be put at approximately 0.8 gram per kilo when given by the mouth.

Further Observations on Vaso-Motor Changes in the Liver. C. W. EDMUNDS, University of Michigan.

The Source of Blood Serum Diastases. L. H. DAVIS (by invitation) AND E. L. ROSS, Northwestern University Medical School.

Efficacy of Adrenaline by Rectum. R. G. HOSKINS, Johns Hopkins University.

Systemic Interaction of Iodine and Thio-Sulphate. HAROLD B. MEYERS AND CHARLES FERGUSON (by invitation), University of Oregon.
Read by title.

THE TOXICITY OF SOME THIOUREAS AND THIURAMDISULPHIDES

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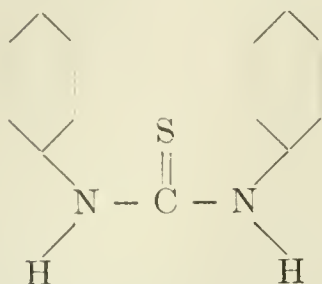
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In connection with the study of some amino compounds, the toxicity of two related thioureas and two related thiuramdisulphides was also ascertained. It is the object of this paper to present briefly the results as to general solubility, lethal dosage and symptoms observed, and correct erroneous impressions gained from the literature concerning diphenylthiourea. As far as we are able to ascertain, the toxicity and actions of the other compounds have not been previously described. These agents are of scientific and toxicological interest. The methods and animals used will be mentioned in connection with the description of each agent in the text. A fatal dose was one that caused death on the day of or following administration.

DIPHENYLTHIOUREA

This is a white crystalline solid, odorless and possessing a distinct bitter taste. It is practically insoluble in hot and cold water, slowly soluble or nearly insoluble in the cold, but readily and completely soluble in the following hot lipoid solvents, olive oil, petrolatum liquidum and kerosene; moderately in cold ether and readily in cold chloroform. The aqueous solution is

neutral to litmus and slightly alkaline to phenolphthalein. The melting point is 153°C. and the structural formula,



According to S. Fraenkel this compound is physiologically inactive, while the phenylthiourea and some methyl and ethylthioureas possess a fair degree of activity. Obviously, the toxicity of diphenylthiourea is not likely to be very high, because of its insolubility in ordinary solvents. Owing to its slight solubility, it was impossible to administer the drug, intravenously, or hypodermically, in aqueous solutions with any degree of success. White rats tolerated up to 0.1 cc. of a saturated aqueous solution per gram, or about 0.1 gram per kilo subcutaneously without any symptoms whatsoever. However, when the agent was administered intragastrically to a cat and rabbits it was found to be active, large doses producing death.

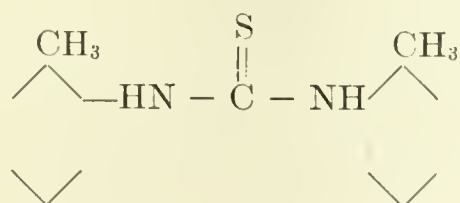
Twelve rabbits received different quantities, ranging from 0.082 to 3 grams per kilo, of the agent directly into the stomach by means of a catheter attached to a glass bulb, using enough water to wash down the drug completely. Some difficulty was encountered in this since the agent is rather light and tends to creep up the sides of glass vessels. The minimal fatal dose was found to be 1.5 grams per kilo. 0.72 gram per kilo administered to a cat in the same way as to the rabbits caused death. The results on a few cats with diphenylthiourea and the other agents reported in this paper indicated that these animals were more susceptible than rabbits, but unfortunately enough cats were not available for this study.

The symptoms in both rabbits and cats consisted of loss of equilibrium and early depression with slowed respiration, ataxia, diarrhea, increased nasal discharge and cyanosis. Later some

increase in excitability and moderate increase in respiratory and pulse rate occurred and, finally, convulsions, coma and death. At autopsy the blood of some animals appeared chocolate colored, suggesting the presence of methemoglobin. From this it is seen that diphenylthiourea is distinctly active when given by stomach, the statement of Fraenkel as to its inactivity to the contrary notwithstanding, although, of course, it can hardly be regarded as a strong poison according to the generally accepted definition in toxicology. Owing to its low lipoid solubility diphenylthiourea does not irritate the skin.

DIORTHOTOLUYLTHIOUREA

As far as we can ascertain, the toxicity and actions of this compound have not been previously described. It is a yellowish amorphous powder possessing a slightly bitter taste, odorless and practically insoluble in cold and hot water; more readily in the following hot, but not cold lipoid solvents; alcohol, kerosene, turpentine, olive oil and liquid petrolatum; slightly soluble in cold ether and readily in cold chloroform. The solubility was less than 1 per cent in hot water and alcohol. Its m.p. is 158°C., and the structural formula,



According to this it differs from diphenylthiourea by being a toluyll compound with the thiourea in the ortho position. From this it might be inferred that the compound is more toxic than diphenylthiourea, but since the activity of a compound may be modified by its complexity and lack of solubility, this conclusion would not necessarily follow. As a matter of fact it was found to be about half as toxic as diphenylthiourea.

Various quantities, ranging from 0.5 to 4 grams per kilo, were administered intragastrically to sixteen rabbits in the same way

as diphenylthiourea described above. The minimal fatal dose was found to be 3 grams per kilo, equivalent to about 180 grams by mouth for a 60 kilo man, which practically places the compound outside the realm of toxicology in the ordinary sense of the word. A cat recovered from 0.5 gram per kilo intragastrically, although moderate symptoms of intoxication were present. The symptoms in rabbits and the cat were similar to those observed with diphenylthiourea described above, and need not be repeated here. White rats survived 0.1 gram per kilo subcutaneously without symptoms.

Diorthotoluyllthiourea, therefore, may be regarded as possessing a very low degree of toxicity. This is quite interesting in view of the fact that the related substance, diphenylthiourea, is more active and toxic. It was not the special object of this study to ascertain the reason for this, but it is suggested that the lesser toxicity of the compound is connected with its different structure (ortho compound), greater complexity and marked insolubility rather than the presence and the position of the methyl groups per se.

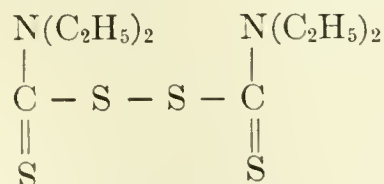
For industrial purposes, where the same objects can be secured by the markedly insoluble and relatively non-toxic diorthotoluyllthiourea, it should be preferred to the more active diphenylthiourea. The danger of deleterious effects from the swallowing of dust of diorthotoluyllthiourea would be greatly reduced. Both the diorthotoluyllthiourea and diphenylthiourea are non-irritating to the skin, permitting the free handling of the agents or manufactured articles containing them without harm.

The effects of the two thioureas described in this paper agree in general with the narcotic and depressant effects of phenylthiourea and several methyl and ethylthioureas previously described by others (cf. S. Fraenkel).

TETRAETHYLTHIURAMDISULPHIDE

This is a white or yellowish crystalline solid with a peculiar aromatic odor resembling violets and possessing a slightly bitter taste. It is insoluble in cold and hot water; slowly in the cold

and easily in such hot lipoid solvents as alcohol, kerosene, turpentine and petrolatum liquidum; somewhat soluble in ether and freely in cold chloroform. It is practically insoluble in hydrochloric acid and sodium hydroxide; melting point is 70°C., and the structural formula is



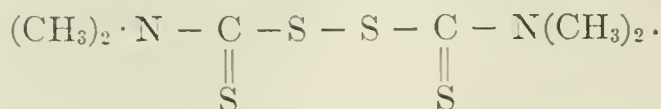
The physiological activity of this compound has not been previously studied.

The results on 9 rabbits and 4 puppies following intragastric administration of the dry agent with a little water, using quantities ranging from 0.3 to 4 grams per kilo, were as follows: The minimal fatal dose was found to be about 3 grams per kilo, equivalent to about 180 grams by mouth for a 60 kilo man. In other words, the compound possesses a very low grade of toxicity. In the fatal cases, no marked symptoms were observed until the following day. The rabbits and dogs that recovered by the end of the second day usually continued to remain healthy. An occasional animal died on the fourth or fifth day, but these fatalities have not been included here as a part of the acute toxicity. On the other hand, the tetramethyl analogue of this compound was found to kill rabbits more rapidly and with much smaller dosage. This will now be described together with the symptoms produced by both compounds, since they were practically identical.

TETRAMETHYLTHIURAMDISULPHIDE

This is a white or yellowish crystalline solid, insipid, odorless and tasteless. It is practically insoluble in cold and hot water; in hydrochloric acid and sodium hydroxide; in cold and hot alcohol, but more easily in hot than cold kerosene, turpentine and petrolatum liquidum and freely in cold chloroform. Its

melting point is 146°, and the structural formula,



The activity of this compound has not been previously described.

Intragastric administration of the dry powder in the usual way to 16 rabbits and 1 cat was found to produce definite symptoms and death with relatively small doses. Doses ranging from 0.08 to 0.7 gram per kilo were administered to the rabbits. The minimal fatal dose was 0.35 gram per kilo. 0.23 gram per kilo was fatal to the cat. According to this the toxicity of this sulphide is about 10 times as great as that of the tetraethylthiuram-disulphide described above. The symptoms produced by both compounds may now be described together. These were characterized by a gradual depression with ataxia, fall of body temperature, slowed respiration and pulse. The depression progressed until finally paralysis of the higher functions and death occurred. Some animals displayed a paresis of the hind extremities which were dragged. The reflexes were markedly depressed and cyanosis was pronounced. Occasionally an animal was seen during the comatose stage with moderately severe, convulsions of asphyxial origin. The effects of subfatal doses were frequently not observed until the end of the second day after administration, while the maximal fatal doses used produced effects somewhat more rapidly. The slowness of action of these compounds is undoubtedly due to slow absorption because of their insolubility in acids and alkalies. At autopsy the only change worthy of note was a marked congestion of the alimentary tract.

Assuming that the solubility was about the same as that of the tetraethyl compound, though this was not definitely ascertained, the greater toxicity of the tetramethyl compound is apparently due to the presence of the methyl groups. This is as would be ordinarily expected, although, as was pointed out above with the two thioureas one of which also contained methyl groups, the risk of such a simple assumption is great. The rôle of the

methyl groups in open chain compounds, as in the case of the thiuramsulfides, might be quite different from their rôle in aromatic compounds. These results serve to emphasize the desirability of investigating all agents or drugs on their own merits before accepting speculative notions or deductions from analogy, and making predictions as to toxicity, actions or therapeutic uses.

Because of their marked insolubility in lipoids, both the ethyl and methyl derivatives are non-irritant to the skin. According to the results obtained, and providing other things are equal, the diethylthiuramsulfide should have the preference over its dimethyl analogue for industrial purposes.

CONCLUSIONS

1. The solubility, toxicity and actions of two related thioureas and two related thiuramdisulphide compounds are reported, three of these compounds having been studied for the first time. The bearing of certain groups on the toxicity of these compounds is discussed briefly.

2. Administered intragastrically to rabbits, diphenylthiourea was found to be physiologically active and toxic, statements in the literature to the contrary notwithstanding. On the other hand, diorthotoluythiourea was found to be only about half as toxic as diphenylthiourea. Cats appeared to be more susceptible to these compounds than rabbits. These agents cause early depression. Later the excitability of higher functions is increased leading to convulsions, coma and death.

3. Tetramethylthiuramdisulphide was found to be about ten times as toxic for rabbits as the tetraethylthiuramdisulphide, which was found to possess a very low grade of toxicity for rabbits and dogs. These agents are marked depressants and act slowly.

4. The results of this study are of toxicologic and industrial interest.

REFERENCE

FRAENKEL, S.: *Arzneimittelsynthese*, 1919, 4th ed.

QUANTITATIVE STUDIES IN CHEMOTHERAPY. V
INTRAVENOUS VERSUS INTRAMUSCULAR ADMIN-
ISTRATION OF ARSPHENAMINE. CURATIVE
POWER AND MINIMUM EFFECTIVE DOSE

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I. THE RELATIVE EFFICACY OF THE INTRAVENOUS AND INTRAMUS-
CULAR ADMINISTRATION OF ARSPHENAMINE AND
NEOARSPHENAMINE

The intramuscular administration of arspfenamine was used exclusively when this drug was first introduced into therapeutics. It was soon found, however, that this procedure leads to a severe reaction at the site of injection, involving great pain and resulting in necrotic changes of a persistent nature. To overcome this difficulty the intravenous injection of the sodium salt was initiated. This method has met with universal use, except in a relatively small number of cases in which difficulties are encountered in introducing a needle into the veins, as in infants and patients with excessive adipose tissue. It was hoped that the introduction of neoarsphenamine would overcome the difficulties of intramuscular injection, for unlike arspfenamine, neoarsphenamine is regarded as a neutral compound which might dissolve directly in the body fluids without the necessity of chemical change. But it was found that even this compound causes a fair degree of irritation, though this irritation may be diminished as in the case of arspfenamine, by the use of an oil suspension instead of an aqueous solution. Inasmuch as the intra-

¹ The authors are indebted to Miss Frère and Miss Dyer for assistance in this investigation.

muscular route is still used for both preparations and because some clinicians, especially Harrison (1) have claimed that this method is more effective than the intravenous, especially in the case of neoarsphenamine, it is a matter of both practical and theoretical interest to establish the relative efficiency of the two methods, a question which cannot be readily answered by clinical observations.

The method we are using for the determination of the standard therapeutic dose of trypanocidal drugs offers an opportunity to attack the problem in a quantitative and experimental way.

The value of any method for the determination of the protozoicidal activity of a drug must in the end be based upon two conditions; first, the possibility of ascertaining through that method, in a rapid and accurate way, the power of the drug to cure the infected animal, and second, the accuracy with which the results so obtained can be translated into the practical problems of human disease. We have previously remarked upon the difficulties inherent in the observation of animals for long periods of time, and the variance of the results based upon such a procedure. We have also pointed out that the method of the minimum effective dose, which we have applied to all the drugs available to us, has the advantage of rapidity and freedom from the complicating factors involved in the criterion of absolute cure; its application to arsenicals of interest in therapy is logically the first step to follow the disclosure of the fundamental principle that these compounds exert a parasitocidal action only after their conversion into the corresponding trivalent oxides (2 and 3). But it remains to be shown just how far this minimum effective dose will go to furnish a reliable index of the curative power of the drug. It has, therefore, been thought advisable to include in this present study sufficient evidence to indicate the *relation between the minimum effective dose and the curative dose*. The *minimum effective dose has been defined as that dose which will just clear the blood stream of parasites in twenty-four hours* (2).

The former threshold figure owes its value in part to the independence of the method of the many factors which render observation for a long period of time difficult. It has been our expe-

rience that a relatively small proportion of animals will survive a long period of time after infection with trypanosomiasis and subsequent treatment, despite the fact that they may not at any time previous to or at the time of death show signs of relapse. Consequently we feel that it is necessary in formulating a procedure for the determination of a curative index to consider as the essential end-point the definite reappearance of trypanosomes in the blood stream. We have accordingly divided our data upon a basis of two conditions:

1. Animals suffering a relapse and showing trypanosomes in the blood stream previous to or at time of death.
2. Animals surviving twenty-eight days without relapse, and animals dying before twenty-eight days *without* trypanosomes in their blood.

We have found that relapses may occur up to twenty-eight days with the strain of *T. equiperdum* used. If an animal survives twenty-eight days without relapse, it may safely be considered as permanently freed of trypanosomes. If an animal dies within the twenty-eight-day period and does not show trypanosomes in the blood stream, its death cannot be attributed to a relapse; the possibility of a relapse has, however, not passed, and it therefore cannot be considered as an "accidental death" or a "survival." A survey of a large amount of data available to us indicates that relapses occur fairly well distributed over the period of two to twenty-five days, and it seems reasonable to assume that an animal dying within the relapse period of twenty-eight days is a partial recovery in proportion to the fraction of that period which it survived. In this sense we have considered the animals which never showed a relapse not in terms of complete survival, but of partial survival based upon the twenty-eighth day period. Take for example, a rat which dies without relapse on the fourteenth day; this is a 0.50 survival, while one which dies on the thirty-first day is a complete survival or 1.00. In this manner all deaths are distributed pro-rata in terms of the twenty-eight-day period during which relapse is possible.

The minimum lethal dose and minimum effective dose of the preparations studied when the drugs were injected intravenously, were as follows:

	CUBIC CENTIMETERS OF 1:100 ARSENIC EQUIVALENT SOLUTION PER KILO	
	Minimum lethal dose	Minimum effective dose
Arsphenamine	75.00	2.0
Neoarsphenamine	100.00	4.0

The arsphenamine for intravenous injection was used as the disodium salt, and for the intramuscular injections, which were made into the muscles of the hind leg, arsphenamine (dihydrochloride) and neoarsphenamine were emulsified in olive oil. The results have been condensed into tables 1 and 2, which need no further explanation.

The most surprising feature about this comparison is the fact that in neither case is the effectiveness any lower when the drug is given intramuscularly than when given intravenously; with arsphenamine it is certainly slightly greater.

The relative effectiveness of the two methods of administration from the standpoint of the minimum effective dose alone is brought out by the data contained in table 3. As will be seen from this table, the rate of disappearance of the parasites from the blood is practically the same in both cases.

In the study of the two typical directly acting compounds, antimonyllactate and "arsenoxide," we pointed out that a dose 50 per cent below the minimum effective dose has practically no effect whatever. In determining this minimum effective dose by the intravenous route, the drug is poured into the blood stream en masse, so to speak, and though a maximum concentration is attained in the blood immediately, it must decrease with great rapidity through absorption of the drug by the tissues, excretion and oxidation to the inert pentavalent type. Attention was at that time called to the fact that the minimum effective dose contained these "elimination" factors and that a general relation between the size of the dose and the rate of destruction of

TABLE 1
Arsphenamine, H54

INTRAVENOUS						INTRAMUSCULAR					
Dose per kilo	Rat num- ber	Died with relapse	Died without relapse	Surviv- ing 28 days	Per- centage surviv- ing	Dose per kilo	Rat num- ber	Died with relapse	Died without relapse	Surviv- ing 28 days	Per- centage surviv- ing
<i>cc. As/100 solution</i>						<i>cc.</i>					
1.278	300	3				1.0	49	2			
	301	1					50	2			
	302	2					51	2			
	303	3					52	2			
	304	3					53	3			
	305	3					54	3			
	306	3					89		26	0.93	
							90		2	0.07	
2.0	9	10					95		S	1.00	
	10	11					175		3	0.11	
	11		S	1.00			176	5			
	12	13					178	4			
	13	11									
	14	19									
	31	27									
	32	7									
3.0	33	8				3.0					
	34	27									
	35	7									
	36	1									
	32A	26									
	33A		19	0.68							
	34A		21	0.75							
	35A		27	0.97							
3.0	36A	12									
	37A		S	1.00							
	1	4									
	2	4									
	3	4									
	4	4									
	5	4									
Total	7			0.00	0.00	Total	12			2.11	17.5
2.0	9	10				2.0	92		S	1.00	
	10	11					93		S	1.00	
	11		S	1.00			94		S	1.00	
	12	13					172	19			
	13	11					173	19			
	14	19					174	9			
	31	27					37	10			
	32	7					38	3			
	33	8					39	3			
	34	27					40	4			
	35	7					41	2			
	36	1					42	2			
Total	12			1.00	8.35	Total	12			3.00	25.0
3.0	32A	26				3.0	43		S	1.00	
	33A		19	0.68			44		S	1.00	
	34A		21	0.75			45		S	1.00	
	35A		27	0.97			46		S	1.00	
	36A	12					47		S	1.00	
	37A		S	1.00			48		S	1.00	
	1	4					55	11			
	2	4					56	27			
	3	4					57		14	0.50	
	4	4					58	11			
	5	4					59	11			

TABLE 1—Continued

INTRAVENOUS						INTRAMUSCULAR					
Dose per kilo	Rat num- ber	Died with relapse	Died without relapse	Surviv- ing 28 days	Per- centage surviv- ing	Dose per kilo	Rat num- ber	Died with relapse	Died without relapse	Surviv- ing 28 days	Per- centage surviv- ing
<i>cc.</i> <i>As/100</i> <i>solution</i>	6	4					60	12			
	7	8					61	8			
	8	4					62	7			
	9	8									
	10	8									
	11	5									
	12		14	0.50							
	Total	18		3.90	21.6	Total	14			6.5	46.5
4.0	81		21	0.75			87		S	1.00	
	82		21	0.75			91		3	0.10	
	83		14	0.50			179	14			
	84		3	0.10			180		S	1.00	
	85		14	0.50			181	18			
	86		14	0.50		4.0	182		S	1.00	
	269		39	1.00			283		5	0.18	
	270		8	0.29			284		12	0.43	
	271	7					286		16	0.57	
	272		18	0.64			287		5	0.18	
							297	13			
							296	7			
	Total	10		5.03	50.3	Total	12			4.46	37.0
6.0	19	9					285		3	0.11	
	20	9					288		7	0.25	
	21	6					289		6	0.21	
	22	13					291		S	1.00	
	23	14				6.0	291		S	1.00	
	24		S	1.00			292		S	1.00	
	273	29					293		S	1.00	
	274		12	0.43			294		5	1.00	
	275		12	0.43			298		S	1.00	
	276		12	0.43			295		S	1.00	
	277		11	0.392							
	278		6	0.21							
	279		10	0.357							
	280	10									
	281		5	0.18							
	282		8	0.29							
	Total	16		3.719	23.24	Total	10			7.57	75.7

TABLE 2
Neoarsphenamine

INTRAVENOUS						INTRAMUSCULAR					
Dose per kilo	Rat num- ber	Died with relapse	Died without relapse	Sur- vivals 28 days	Per- centage surviv- ing	Dose per kilo	Rat num- ber	Died with relapse	Died without relapse	Sur- vival 28 days	Per- centage sur- vival
<i>cc.</i> <i>As/100</i> <i>solution</i>						<i>cc.</i>					
4.5	26		S	1.00		4.5	191		24	0.86	
	27	11					192	19			
	28		S	1.00			193	23			
	29	15					195		S	1.00	
	30		S	1.00			196		S	1.00	
	31	20									
Total	6			3.00	50.0	Total	5			2.86	57.0
6	96	6				6	169		S	1.00	
	97	13					170		12	0.43	
	98		S	1.00			171	15			
	99		S	0.28			197		S	1.00	
	101		S	0.28			198		S	1.00	
	239		16	0.57			199	15			
	240	12					242		26	0.97	
	241		16	0.57			243		7	0.25	
	248		S	1.00			261		9	0.32	
Total	9			3.70	41.0	Total	10			5.26	52.6
9	244		S	1.00		9	251		S	1.00	
	245		19	0.32			252		3	0.11	
	246		S	1.00			253		S	1.00	
	247		S	1.00			254		15	0.54	
	248		S	1.00			255		17	0.61	
	250		S	1.00			256		S	1.00	
	257		S	1.00			263		9	0.32	
	258		S	1.00			264		8	0.29	
	259		S	1.00			265		6	0.21	
	262		S	1.00			266		9	0.32	
Total	10			9.32	93.2	Total	10			5.40	54.0

parasites undoubtedly exists. If the “elimination” factors could be avoided, that is, if the drug were not absorbed and temporarily fixed by the tissues, excreted or oxidized, and the effective concentration available for reaction with the parasites were not thereby diminished, the minimum effective dose would be considerably lowered.

Now in the intramuscular administration of arsphenamine and neoarsphenamine, a depot is established from which the drug is

TABLE 3
The minimum effective dose

INTRAVENOUS						INTRAMUSCULAR						
Rat num- ber	Trypano- some count at time of treatment	Days				Rat num- ber	Trypano- some count at time of treatment	Days				
		1	2	3	4			1	2	3	4	
Arsphenamine												
9	92,000	2000	4000	—	—	92	160,000	—	—	—	—	—
10	117,000	500	—	—	—	93	91,000	—	—	—	—	—
11	178,000	—	—	—	—	94	98,000	—	—	—	—	—
12	153,000	—	500	—	—	172	158,000	—	—	—	—	—
13	217,000	—	—	—	—	173	156,000	—	—	—	—	—
14	101,000	—	2000	—	—	174	143,000	—	6000	43,000	269,000	544,000
Neoarsphenamine												
26	187,000	1000	—	—	—	191	318,000	—	—	—	—	—
27	146,000	5000	—	—	—	192	323,000	—	—	—	—	—
28	117,000	1000	—	—	—	193	295,000	—	—	—	—	—
29	144,000	1500	—	—	—	195	183,000	—	—	—	—	—
30	184,000	1000	—	—	—	196	156,000	—	—	—	—	—
31	151,000	3000	—	—	—							

removed only slowly and since it cannot oxidize in olive oil, this depot will maintain a *low concentration* of active arsenic in the blood for a *long period* of time. The net result is, contrary to what one would expect from reasoning from the behavior of antimonyllactate and “arsenoxide,” slightly in favor of the intramuscular route.

A comparison of the *survivals as an index of the curative value* shows that with neoarsphenamine, there is little choice between

the two methods of injection. The net result clearly shows that the two methods of administration are at least equally efficacious in rat trypanosomiasis, and indicates in a preliminary way that the intramuscular may be superior to the intravenous route so far as arsphenamine is concerned.

If the intramuscular administration of arsphenamine or neoarsphenamine is just as effective as the intravenous method of treatment in rat trypanosomiasis, which is predominantly a blood infection, it is probable that the same thing will be true for the treatment of syphilis. The differences in the distribution of the parasites in the two diseases is one of degree only, and fundamental relations of the nature disclosed by this study must apply, qualitatively at least, to both cases. This evidence has, therefore, some bearing on the relative efficiency of intramuscular and intravenous injections of arsphenamine and neoarsphenamine in the treatment of syphilis. By the intramuscular injection of the drug, we are bringing rat trypanosomiasis into line with syphilis by simulating at least one condition essential to the treatment of the latter disease—prolonged retention of the arsenic in the body. By judging the trypanocidal activity under the condition of absorption and elimination attending intramuscular injection, the relative value of arsphenamine and neoarsphenamine for exerting this prolonged parasitocidal action is made evident. Under these conditions as we have seen, arsphenamine shows up better than neoarsphenamine.

The work of Kolle, Hartoch, Rothermundt and Schürmann (4) with insoluble antimony preparations is especially interesting in this connection. They studied the trypanocidal effect of a large number of antimony preparations on mice infected with Nagana, but obtained the best results with antimony trioxide, a substance practically insoluble in water, which they administered by intramuscular injection of an oil suspension. They found a therapeutic ratio of 1:100 and noticed that even when the mice were heavily infected, the parasites disappeared in twenty-four hours. They obtained similar results with metallic antimony, but the therapeutic ratio was 1:10. They also tried injections of the metallic antimony and other insoluble compounds and demon-

strated a marked therapeutic action without any toxic effects. That such relatively insoluble substances should exert so marked a therapeutic action is evidence that assimilation by the body fluids takes place with remarkable rapidity, and that *minute quantities of the drug will exert a cumulative toxic effect upon the parasites if maintained for a long period of time.*

The fact that the intravenous and intramuscular administration of arsphenamine and neoarsphenamine are, to say the least, equally efficient from a therapeutic standpoint is also supported by the observations of Harrison (1). Based on careful observations of the treatment of cases of primary and secondary syphilis by means of intramuscular and intravenous injections of neoarsphenamine, this observer comes to the conclusion that the intramuscular method of administration is superior in bringing about an immediate therapeutic effect.² The spirochetes disappear from the lesions just as rapidly after the first intramuscular as after the first intravenous injection, and the Wassermann reaction is more quickly influenced. A most significant observation made by Harrison was that the general reaction which followed the intramuscular injections was much less than after the intravenous. He states that he had not experienced any cases of vasomotor disturbance—flushing, constriction of the throat and chest, etc. This would be logically expected, for pharmacological reasons, as with intravenous injections the whole dose is thrown into the blood all at once, whereas given intramuscularly the absorption is gradual and would therefore reduce the possibility of acute reactions.

After the completion of this work, Fordyce and Rosen (5) published their results obtained in the treatment of congenital syphilis by means of intramuscular injections of neoarsphenamine (dissolved in water) and mercuric chloride (in oil). They point out the difficulties encountered in the treatment of syphilis in infants and call attention to the danger of the present methods (injection into the longitudinal sinus, the veins of the arm, scalp

² See also Craig, C. F., *The Wassermann Test*. 1918, 180. C. V. Mosby Company, St. Louis.

or neck, or intragluteal injections) in the hands of clinicians. With their technic they observed no complications from the intramuscular injection of neoarsphenamine and the improvement in the clinical condition of the patients was very gratifying.

Practical bearing. Having shown that all the available evidence, both experimental and clinical, bears out the therapeutic efficiency of the intramuscular administration of arsphenamine and neoarsphenamine, it is well to emphasize the practical significance of these observations. It will be granted that intravenous drug administration in the clinic is generally regarded as a last resort, when for various reasons the drug cannot be given by other routes. In the case of arsphenamine and its substitutes, it was largely on account of the local irritation that the intramuscular method was abandoned in favor of the intravenous. Should it be possible, however, to reduce this local irritation, and there are reasons to believe that this can be accomplished when neoarsphenamine is used, then the intramuscular injection of this drug in the treatment of syphilis should be given preference over the intravenous injection, on account of the simplicity of the technic and the probability of reducing the number of acute toxic reactions. The latter represent probably the most alarming feature which the syphilologist has to contend with, and from a clinical point of view are far more troublesome than a mild degree of muscular irritation involving some slight pain. Work is therefore in progress in this laboratory with a view to elaborating a preparation of neoarsphenamine which may be devoid of irritating properties.

II. THE CURATIVE POWER IN TERMS OF THE MINIMUM EFFECTIVE DOSE

This part is essentially an extension of some of the principles discussed in part I of this paper, to compounds other than the arsphenamines.

For the purpose of determining the curative power of these arsenicals, multiples of the minimum effective dose were injected intravenously in the form of the sodium salts. The animals

TABLE 4

MINIMUM EFFECTIVE DOSE	RAT NUMBER	DIED WITH RELAPSE	DIED WITHOUT RELAPSE	SURVIVALS	PERCENTAGE SURVIVALS
<i>p</i> -aminophenylarsenious oxide					
1	332	12		0.00	
	1 S	3		0.00	
	2 S	5		0.00	
	6 S	8		0.00	
Total.....	4			0.00	0.0
1.5	300	23		0.00	
	3 S	3		0.00	
	4 S	5		0.00	
Total.....	3			0.00	0.0
<i>Phenyl glycine-p</i> -arsonic acid					
1	113	12		0.00	
	114		4	0.14	
	115		3	0.11	
Total.....	3			0.25	8.3
1.5	116	2		0.00	
	117		3	0.11	
	118		3	0.11	
	233		2	0.07	
Total.....	4			0.29	5.8
2	216		3	0.11	
	214		3	0.11	
	232		3	0.11	
	268		3	0.11	
	267		3	0.11	
	350	2		0.00	
	351		3	0.11	
	352		3	0.11	
	353		3	0.11	
	215	11		0.00	
Total.....	10			0.88	8.8

TABLE 4—Continued

MINIMUM EFFECTIVE DOSE	RAT NUMBER	DIED WITH RELAPSE	DIED WITHOUT RELAPSE	SURVIVALS	PERCENTAGE SURVIVALS
<i>Atoxyl</i>					
1	107	11		0.00	
	108	13		0.00	
	109	13		0.00	
	110	24		0.00	
	111	11		0.00	
	112	15		0.00	
	344	15		0.00	
	345	15		0.00	
	346	15		0.00	
	347	10		0.00	
Total	10			0.00	0.0
1.5	218		7	0.25	
	219		10	0.36	
	220		7	0.25	
	221		7	0.25	
	222		7	0.25	
	223		8	1.00	
Total	6			2.36	39.0
2	217		11	0.39	
	234	14		0.00	
	235		13	0.46	
	236		9	0.32	
	237		9	0.32	
	238		8	1.00	
Total	6			2.49	41.5
3	1		3	0.10	
	2		1	0.03	
	3		4	0.14	
	4		5	0.17	
	5		5	0.17	
	6		17	0.60	
	7	8		0.00	
	8	7		0.00	
	9	8		0.00	
	10	8		0.00	
Total	10			1.21	12.1

TABLE 4—Continued

MINIMUM EFFECTINE DOSE	RAT NUMBER	DIED WITH RELAPSE	DIED WITHOUT RELAPSE	SURVIVALS	PERCENTAGE SURVIVALS
<i>Phenylarsenious oxide</i>					
1	20	4		0.00	
	21	5		0.00	
	22	5		0.00	
	23	5		0.00	
	24	5		0.00	
	25	3		0.00	
Total.....	6			0.00	0.00
1.5	44	4		0.00	
	45	4		0.00	
	46	5		0.00	
	47	4		0.00	
	48	11		0.00	
Total.....	5			0.00	0.00
2	166	5		0.00	
	167	4		0.00	
	168	10		0.00	
	200	6		0.00	
	201	6		0.00	
	202	6		0.00	
Total.....	6			0.00	0.00
3	186	3		0.00	
	183	11		0.00	
	184	7		0.00	
	185	10		0.00	
	367	11		0.00	
	368	11		0.00	
	369	11		0.00	
	370		4	0.00	
	371	11		0.00	
	372	11		0.00	
Total.....	10			0.00	0.00

TABLE 4—*Continued*

MINIMUM EFFECTIVE DOSE	RAT NUMBER	DIED WITH RELAPSE	DIED WITHOUT RELAPSE	SURVIVALS	PERCENTAGE SURVIVALS
<i>m-amino-p-oxyphenylarsonic acid</i>					
1	61	6		0.00	
	62		S	1.00	
	63	9		0.00	
	64		S	1.00	
	65	22		0.00	
	66	6		0.00	
Total.....	6			2.00	33
1.5	67		S	1.00	
	68		10	0.36	
	69		S	1.00	
	70	13		0.00	
	224		6	0.21	
	225		6	0.21	
Total.....	6			2.78	46.5
2	228		7	0.25	
	229		7	0.25	
	230		9	0.32	
	226		9	0.32	
	227		S	1.00	
	231	11		0.00	
Total.....	6			2.14	35.6
3	7		14	0.50	
	8		10	0.36	
	9		1	0.03	
	10	19		0.00	
	11		7	0.25	
	12		5	0.17	
Total.....	6			1.31	21.8
<i>N-phenyl glycine amide-p-arsonic acid</i>					
1	56	6		0.00	
	55	10		0.00	
	53	10		0.00	
	54	10		0.00	
Total.....	4			0.00	0.00

TABLE 4—Continued

MINIMUM EFFECTIVE DOSE	RAT NUMBER	DIED WITH RELAPSE	DIED WITHOUT RELAPSE	SURVIVALS	PERCENTAGE SURVIVALS
<i>N-phenyl glycine amide-p-arsonic acid—continued</i>					
1.5	57	6	10	0.36	
	58		7	0.25	
	59		9	0.32	
	60			0.00	
Total.....	4			0.93	23.2
2	119	11	S	0.00	
	120	6		0.00	
	121	21		0.00	
	203	18		0.00	
	204			1.00	
	205	18		0.00	
Total.....	6				16.6
3	151	6	10	0.36	
	152			0.00	
	153		20	0.72	
	154		S	1.00	
	206		19	0.68	
Total.....	5			2.76	55.0
<i>m-amino-p-oxyphenylarsenious oxide, "arsenoxide"</i>					
1	17	9		0.00	
	18	6		0.00	
	15	9		0.00	
	19	12		0.00	
	16	16		0.00	
Total.....	5			0.00	0.00
1.5	39	13	14	0.50	
	42			0.00	
	38		S	1.00	
	40		S	1.00	
	41		S	1.00	
	43		S	1.00	
Total.....	6			4.50	75.0

TABLE 4—*Concluded*

MINIMUM EFFECTIVE DOSE	RAT NUMBER	DIED WITH RELAPSE	DIED WITHOUT RELAPSE	SURVIVALS	PERCENTAGE SURVIVALS
<i>m-amino-p-oxyphenylarsenious oxide, "arsenoxide"—continued</i>					
2	102		8	0.29	
	106		5	0.18	
	103		13	0.46	
	105		7	0.25	
	104		S	1.00	
Total.....	5			2.18	43.5
3	1		S	1.00	
	2		25	0.89	
	4		4	0.14	
	5		7	0.25	
	6		25	0.89	
	8		15	0.53	
Total.....	6			3.70	61.6
<i>Arsacetine</i>					
1	74	16		0.00	
	75	5		0.00	
	76	5		0.00	
Total.....	3			0.00	
1.5	77	15		0.00	
	78	11		0.00	
	79	19		0.00	
	80	14		0.00	
	213		S	1.00	
Total.....	5			1.00	20.0
2	207	15			
	208		26	0.93	
	209		S	1.00	
	210		21	0.75	
	211		S	1.00	
	212		S	1.00	
Total.....	6			4.68	78.0
3	318		S	1.00	
	319		S	1.00	
	320		S	1.00	
	324	86*		0.00	
	325		S	1.00	
	326		S	1.00	
Total.....	6			5.00	83.5

* The blood of this rat showed trypanosomes during the entire period of observation, a fact which may perhaps be explained by the drug having reduced the virulence of the strain.

TABLE 5
Relation of empirical therapeutic ratio $\frac{M.L.D.}{M.E.D.}$ to curative power

DRUG	$\frac{M.L.D.}{M.E.D.}$	PERCENTAGE SURVIVAL OF TREATED ANIMALS		
		1 M.E.D.	2 M.E.D.	3 M.E.D.
p-aminophenylarsenious oxide.....	2	0		
Phenylglycine-p-arsonic acid.....	2	8.3	8.8	
Atoxyl.....	4	0.0	41.5	12
Phenylarsenious oxide.....	5	0.0	0.0	0
m-amino-p-oxyphenylarsonic acid.....	6	33.0	35.6	21
N-phenylglycine amide-p-arsonic acid...	10	0.0	16.6	55
m-amino-p-oxyphenylarsenious oxide...	13	0.0	43.5	61
Arsacetine.....	20	0.0	78.0	83

were kept under observation for at least sixty days, though they were considered as complete survivals after twenty-eight days. Tables 4 and 5 present the results obtained in this study.

It will be seen from these tables that animals treated with p-amino-phenylarsenious oxide or phenylarsenious oxide never survived the twenty-eight-day period. The reason for this is that the minimum lethal dose so closely approaches the minimum effective dose that even though the animal may be rendered sterile, the combined shock of trypanosomiasis and drug injection is too great to be survived. A notable exception in the case of compounds of this type is m-amino-p-oxyphenylarsenious oxide, an oxidation product of arsphenamine, which shows a fairly high curative power and also has a more favorable empirical therapeutic ratio $\left(\frac{M. L. D.}{M. E. D.} = 13\right)$. The curative power of the pentavalent arsenicals studied (phenyl glycine-p-arsonic acid, atoxyl, m-amino-p-oxyphenylarsonic acid and arsacetin) shows good agreement with the empirical therapeutic ratio, although there is no indication of a simple proportion. The data relating to arsphenamine and neoarsphenamine, incorporated in the first part of this paper, also show that there is sufficient general agreement with the ratio $\frac{M. L. D.}{M. E. D.}$, so that this may serve as an index of the therapeutic efficiency of a compound. We real-

ize, however, that the latter method cannot fully replace the tedious observation of chronic types of infections, although we are not aware of any evidence which would indicate that there is an essential difference in the results obtained by the two procedures. Certain phases of the chemotherapeutic action of arsenicals, as for example the treatment of neurosyphilis, will have to be worked out with methods especially designed for this purpose.

SUMMARY

1. The intramuscular administration of arsphenamine and neoarsphenamine is just as efficacious as the intravenous administration of these drugs in the treatment of experimental trypanosomiasis, as judged by both the minimum effective dose and the percentage of survivals of treated animals. The significance of this point in the treatment of human syphilis is discussed.

2. The ratio of the minimum lethal dose to the minimum effective dose, $\frac{M. L. D.}{M. E. D.}$, is a substantial index to the curative power of a given drug under experimental conditions.

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THE ACTION OF DRUGS IN INFECTION

I. THE INFLUENCE OF MORPHINE IN EXPERIMENTAL SEPTICEMIA

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Because of its extensive use, the therapeutic value of opium and its derivatives, in infectious diseases, should be thoroughly understood by the internist and surgeon. The influence of morphine on infection is apparently not generally understood, although as early as 1898 Cantacuzene (1) called attention to the fact that phagocytosis is inhibited by its administration. Several years later, Reynolds (2) verified the above experiment and investigated the problem further. He found that the phagocytic property of leucocytes towards staphylococcus aureus was markedly inhibited. Later (1913) the influence of drugs on infection was studied by Arkin (3) who noticed that phagocytosis was hindered by morphine solutions in vitro. Cantacuzene (4) found that animals given various derivatives of opium succumbed more readily to anthrax and other infectious diseases than did normal animals. Crothers (5) states that addicts of morphinism are very often subjects of and succumb to, pneumonia, nephritis, and other diseases.

The purpose of the present investigation was to observe the course of septicemia produced by the *Streptococcus hemolyticus* as influenced by morphine and to compare the course of the infection in morphinized, and non-morphinized animals.

METHODS

Twelve healthy rabbits, weighing about 2.5 to 3 kilos each were used. The strain of streptococcus used was isolated from a case of human septicemia terminating fatally. Two of the twelve rabbits were used as controls: Each of these were injected subcutaneously with 0.030 gram (0.5 grain) of morphine sulphate in 1 cc. water, per kilo of weight. Five of the remaining rabbits were likewise injected with an equal amount of morphine sulphate per kilo of weight and also with a twenty-four hour blood-agar culture of *Streptococcus hemolyticus* suspended in normal saline. The remaining five rabbits were injected with an equal amount of streptococcus culture per kilo of weight, as those above; however, they were given no morphine.

A second similar experiment to the above was done with a series of ten rabbits. Two animals received morphine only; three received streptococci only; and five received both morphine and streptococci. The strain of hemolytic streptococcus used in this series was isolated from a case of tonsillitis. Thinking that a larger dose of morphine might prove fatal to normal rabbits, the two controls (I and XI) were given 0.03 gram (0.5 grain) at the beginning of the experiment; a second dose fifteen hours later, and a third dose forty-five hours later. Numbers IV, V, VI, VII, VIII, those receiving both streptococci and morphine, were likewise injected with morphine as the control animals.

Observations of the first series were recorded every six hours (see table 1). Observations of the second series were recorded every three hours (see table 2).

We also observed that the temperature of the rabbits, receiving the morphine, was lowered from 0.5° to 1.5° and progressively came back to normal gradually as the effects of the morphine disappeared (10). This is probably due to the quieting influence of the drug as well as a central antipyretic action. The temperature was likewise lowered in those rabbits receiving both morphine and intravenous injection of streptococci; however, in these the temperature began to rise before the effects of the morphine disappeared.

Post mortem examinations were made on all animals succumbing to the infection. The organisms were recovered from the heart blood in every instance; this gave evidence that infection was present.

TABLE 1

		8.00 A. M.	2.00 P. M.	8.00 P. M.	2.00 A. M.	8.00 A. M.	2.00 P. M.	8.00 P. M.	2.00 A. M.
Controls: 0.03 gram morphine sulphate per kilo									
V	Injected	+++	++	++	+	—	—	—	—
VI	Injected	+++	++	+	+	—	—	—	—
Morphine as above plus streptococci									
I	Injected	+++	+++	++	+++	Dead			
IV	Injected	+++	+++	++	+++	Dead			
VII	Injected	+++	+++	++	+++	Dead			
VIII	Injected	+++	+++	++	+++	Dead			
IX	Injected	+++	+++	++	+++	Dead			
Streptococci as above, no morphine									
II	Injected	+	+	++	++	+	+	—	—
III	Injected	+	+	++	++	+	+	—	—
X	Injected	—	+	++	+	+	—	—	—
XI	Injected	+	++	++	++	+	+	+	+
XII	Injected	+	+	++	++	+	—	—	—

Note: The signs “— and +” indicate the condition of the animal with respect to activity, attitude and reaction toward stimuli. (—) signifies that the animal seems as if it had never been touched. (+) signifies the variation from normal.

DISCUSSION

In the administration of morphine at least two viewpoints should be considered; viz., the value of morphine as a sedative, and the effect it has in lowering the defense of the body against infection. That morphine usually produces a depression of the senses, insensibility to pain, and is often followed by comfort and rest can hardly be questioned, especially when it is administered in proper dosage. Its value in this respect is excellent and is, for this reason appreciated by the patient and those giving the drug. One is undoubtedly often justified in its use

TABLE 2

		STREPTOCOCCUS-MORPHINE					STREPTOCOCCUS			MORPHINE	
		IV	V	VI	VII	VIII	III	IX	X	I	XI
4 p.m.	1-22	*	*	*	*	*	*	*	*	*	*
7 p.m.		+++	+++	+++	+++	+++	—	—	—	+++	+++
10 p.m.		+++	+++	+++	+++	+++	—	—	—	+++	+++
1 a.m.	1-23	++	++	+	++	++	—	—	—	++	++
4 a.m.		+	++	++	+++	++	+	—	—	++	+
7 a.m.		+++	+++	+++	+++	+++	+	+	—	+++	+++
10 a.m.		+++	+++	+++	+++	+++	+	+	+	++	++
1 p.m.		++	Dead	+++	+++	+++	+	+	+	+	++
4 p.m.		++		+++	Dead	++	+	+	+	+	+
7 p.m.		++		+++		++	++	+	+	+	+
10 p.m.		++		Dead		++	++	+	+	—	+
1 a.m.	1-24	++				++	++	++	+	—	—
4 a.m.		++				++	++	++	+	—	—
7 a.m.		++				++	++	++	+	—	—
10 a.m.		++				++	++	++	+	—	—
1 p.m.		+++				+++	++	++	++	+++	+++
4 p.m.		+++				+++	++	++	++	++	++
7 p.m.		+++				+++	+++	++	++	+	+
10 p.m.		+++				+++	+++	++	++	—	—
1 a.m.	1-25	++				+++	+++	++	++	—	—
4 a.m.		++				+++	+++	++	++	—	—
7 a.m.		++				+++	+++	++	++	—	—
10 a.m.		+++				+++	+++	++	++	—	—
1 p.m.		+++				+++	+++	++	++	—	—
4 p.m.		+++				Dead	+++	++	++	—	—
7 p.m.		+++					+++	++	++	—	—
10 p.m.		+++					+++	++	++	—	—
1 a.m.	1-26	+++					++	++	++	—	—
4 a.m.		+++					++	++	++	—	—
7 a.m.		+++					++	++	+++	—	—
10 a.m.		+++					+++	++	+++	—	—
1 p.m.		Dead					+++	+++	+++	—	—
4 p.m.							+++	+++	++	—	—
7 p.m.							+++	+++	++	—	—
10 p.m.							Dead	+++	+	—	—
1 a.m.	1-27							+++	+	—	—
4 a.m.								Dead	—	—	—
7 a.m.									—	—	—

* Animals were injected at this time. All injections were proportionate to weight.

whenever infection is not involved. Morphine is also valuable in inhibiting excessive bowel movements; but when it is administered in the presence of infection, the greatest precaution should be taken. One should know exactly why it is given, its effect on the patient, and of equal importance, its effect on the course of the infection. Only after the nature, severity of the infection, its ultimate outcome, and the symptoms of the patient, are considered, can a proper decision be made regarding the value of morphine. Many surgeons object to the use of morphine, stating that its effects modify or entirely obscure important symptoms. They are apparently justified in making such an assertion. For instance, in acute appendicitis, many of the diagnostic symptoms are either greatly modified or entirely eliminated. In this and other cases morphine should be withheld until the diagnosis is made. However, just as important a factor to be considered is the effect of morphine on the course of infection. It is in all probability true that some of the fatal terminations of infection where morphine has been used would not have resulted had the drug been withheld.

Crothers (6), after a thorough study of morphinism, states that few morphine habitués live longer than ten to fifteen years after the beginning of the addiction; and, most of them die in about ten years. He also states that the continuous use of morphine without break or change makes the case a fatal one, death following from exhaustion and acute intercurrent diseases.

Elsner (7), in writing on prognosis of morphinism says, "Most of my cases of morphinism have fallen into wretched states, have developed intercurrent disease—often infection." He also says that pyemia, tuberculosis, and pneumonia are complicating conditions. Sollmann (8) states that opiophagic diabetics die sooner than others.

The lowering of temperature in the morphinized animals may have been due either to the quiet condition produced, or a central action or to both. In doses large enough to produce narcosis, there undoubtedly is a depression of the bodily metabolism, with consequent depressions of oxidation and heat production. Cushny (9) states that morphine frequently causes a fall in the

temperature which may be explained by the less active movements and the dilatation of the cutaneous vessels; the temperature regulating center is also affected.

Gottlieb (10) observed that a rise in temperature due to heat puncture of brain could be lowered by the administration of morphine as well as by antipyretics. This he accomplished by small doses (0.01 to 0.02 gram for rabbits weighing about 1900 grams; in a few instances he repeated, making a total of 0.03 gram in four hours). In our work, 0.03 gram per kilo, was used—a somewhat greater dose than that used by Gottlieb.

From the results obtained by us in this experiment, it is clear that morphine in itself was not fatal for the animals, in as much as about one-tenth of the fatal dose of morphine sulphate for rabbits was given (according to Sollmann (11)). Those animals receiving both morphine and streptococcus (hemolytic) injections, did not come out of the stupor at all, but soon succumbed. Those animals receiving *Streptococcus hemolyticus* injections only, gradually showed increased symptoms of the septicemia and then the symptoms of the immediate infection declined. After ten to fifteen days, however, these animals acquired complications, such as arthritis, snuffles, or other diseased condition which caused their death.

It is natural for the body to protect itself against infection, and to rid itself of the infection after it has once become invaded by micro-organisms. This it does in various ways, amongst which are phagocytosis, elimination, bodily nutrition, elevation of temperature, and specific antibodies. Then, since the various factors mentioned above are true, a depression, elimination of any one or several of the factors will allow infection to proceed more rapidly and extensively. As mentioned above, morphine in certain concentrations hinders phagocytosis; and produces intestinal stasis, which hinders elimination and allows the organisms of the gastro-intestinal tract to produce toxic products that are taken into the general system, thus adding poisonous material to that which the par-enteral infection produces.

Arkin (12) states that morphine should be used cautiously in bacterial disease. Hewlett (13) states that, from the results

of studies upon animals we must conclude that a moderately high temperature, even though maintained for a long time, is not in itself a dangerous manifestation. On the whole, an increased temperature causes artificial infections in animals to run a more favorable course and it increases the speed with which protective antibodies are formed. According to Rolly (14) the favorable effect of higher body temperatures on the formation of antibodies exists up to temperatures of 103.2° to 104° F.

The recent work of Emil Diehl (15) on collapse poisons (amylene hydrate, antifebrine, dysentery toxin, etc.) has some bearing on this problem. He points out that the difference between antipyretics and collapse poisons is mainly one of degree. The toxic dose of collapse poisons being very close to any therapeutic effect they might have. Very large doses of antipyretics also may have a collapse effect. In collapse he found that the regulatory mechanism which prevented undercooling of the body is *lost* while the protection against over-heating remains. The collapse animal is very much like a poikilothermal animal. Consequently when exposed to cold temperature the animal readily succumbs. Among his collapse poisons was dysentery toxin. One might suppose that the action of hemolytic streptococcus toxin is similar to this toxin, and that the morphine aided in the collapse. The amount of morphine used however was not per se sufficient to cause collapse, yet the room temperature (about 20°) may have had some influence on the last stages of the infection, since in some cases Diehl found that the lowest temperature adjustments of the body to cooling external temperature could take place within a very narrow range not below 30° to 34°C. This point however is of little bearing on our present problem since we were working with normal external temperatures, such as would prevail in a home or hospital. The conclusion of Diehl that the action is mainly central, we think would hold also for hemolytic streptococcus, though future work may somewhat modify this opinion.

Since alcohol acts at times somewhat as an antipyretic, it is perhaps proper here to refer to Laitinen (16) who observed that alcohol diminishes the resistance to bacterial infection.

Furthermore, while the animal is under the influence of morphine, it does not eat normally, if at all, and metabolism in

general is at a standstill or is depressed; and in consequence of this, interpretation of experimental or practical observations deserve careful consideration. Further observations are being recorded and will appear in other papers of the series.

CONCLUSIONS

1. Morphine sulphate given in 0.03 gram (0.5 grain) doses which is about one-sixth to one-tenth fatal dose lowers the resistance of rabbits toward septicemia produced by the *Streptococcus hemolyticus*.

2. Morphine sulphate, given as above, lowers the temperature of rabbits.

3. In the administration of morphine at least two effects should be considered: First, the sedative action of morphine; second, the influence of morphine on the course of infection.

4. The harmful influence of morphine is probably due to a number of factors, such as inhibition of phagocytosis, increase in intestinal stasis, with the increased production of toxins, and a general depression of the body temperature, of metabolism and the body defense.

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THE SALICYLATES

XIII. THE LIBERATION OF FREE SALICYLIC ACID FROM SALICYLATE IN THE CIRCULATION

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A study of the liberation of free salicylic acid from salicylate in the blood is of interest in connection with the explanation of cardiac complications accompanying rheumatic fever, including the difference in the susceptibility to injury (functional or morphological or both) of the mitral and tricuspid valves. It is said that these complications and valvular differences exist also in the absence of salicylate therapy, and, therefore, there could be no causal relation between salicylate effect and occurrence of valvular endocarditis. However, it is probable that the majority of individuals susceptible to or suffering from rheumatic symptoms have received salicylate in some form at some time during their attacks. Therefore, it is conceivable that some relation between the valvular changes and salicylate effect might exist.

The following theory has been advanced by some, namely, that the absence of a right-sided valvular endocarditis is due to the antiseptic qualities of free salicylic acid liberated here by virtue of the greater carbon dioxide content of the venous blood. The occurrence of mitral endocarditis, according to this view, would be associated with the greater alkalinity of the arterial blood, which cannot liberate free salicylic acid, and consequently does not possess antiseptic qualities. Although this view appears hardly compatible with the modern conceptions of the mechanism of neutrality regulation of the blood, yet it was thought

desirable to put it to a test. This was done on animals, injected with salicylate and subjected to the most favorable conditions for reduction of the alkalinity of blood, mainly by means of fatal asphyxia. In this connection opportunity was taken to test the claims of Poulsson (1), namely, that free salicylic acid is demonstrable (by ether extraction) in the blood of asphyxiated, but not in normal animals treated with salicylate. From this, and since inflamed tissues contain more CO_2 than normal tissues, Poulsson argues that the conditions in rheumatic joints are favorable for the liberation of salicylic acid. As for cardiac complications, Poulsson considers treatment with salicylate to have no influence, since the CO_2 tension in the heart is too low for the liberation of free salicylic acid.

The degree of acidity necessary for the liberation of free salicylic from salicylate in vitro was also ascertained. These results will be presented first.

DEGREE OF ACIDITY REQUIRED FOR THE LIBERATION OF FREE SALICYLIC FROM SALICYLATE IN VITRO

This was ascertained by extraction of mixtures of sodium salicylate and pure "buffer" solutions, and mixtures of salicylate and "buffers" and beef serum and plasma with immiscible solvents. The final concentration of salicylate in all the mixtures was 0.05 per cent, equivalent to more than twice the concentration occurring in blood of patients, receiving full therapeutic doses of the drug in rheumatism. The final concentration of serum and plasma in the mixtures containing these constituents was 25 per cent. The acidity of the mixtures was altered by means of the ordinary phosphate "buffer" solutions, and dilute hydrochloric acid for the higher acidities, and estimated colorimetrically in the usual way. Phenolsulphonephthalein was used for the range from $\text{pH} = 6.6$ to 8.4 and rosolic acid for $\text{pH} = 4.9$ to 6.4 . The immiscible solvents used were ether, chloroform and petroleum ether (boiling point $30^\circ\text{C}.$). Petroleum ether was found to be most suitable because of the low coefficient of solubility of water and serum in it.

In detail the experiments were carried out as follows: To 10 cc. of a 0.1 per cent solution of salicylate in each separatory funnel were added 10 cc. of the "buffer" solution and 15 cc. of immiscible solvent. In the serum and plasma experiments, 5 cc. of 0.2 per cent sodium salicylate were mixed with 5 cc. of serum or plasma and 10 cc. of the "buffer" solution and 15 cc. of immiscible solvent. The funnels were then gently rotated for one hour. The clear ethereal extracts free from emulsion were now separated, transferred to beakers and allowed to evaporate spontaneously. The residues left after evaporation were treated with 10 cc. of hot water, filtered through cotton into small test tubes, and 3 to 4 drops of 2 per cent ferric alum were added to each for the detection of salicyl, using a 0.05 per cent solution of salicylate as control. After the extractions were completed, the aqueous portions were used, as a rule, for the determination of the final hydrogen-ion concentration of the mixtures colorimetrically as indicated above. Sometimes this was done with separate sets of similar mixtures.

It is desirable to point out certain features about experiments of this sort, which, if overlooked, can easily give false results and lead to erroneous conclusions. It is necessary to prevent contamination of the ethereal extracts with the emulsified portions especially with serum and plasma mixtures, since a mere trace of such emulsified salicylate gives a positive salicyl reaction with ferric alum. At times it was necessary to dehydrate the extracts by calcium chloride. The use of acetic ether (ethyl acetate) as an immiscible solvent is precluded, despite its popularity among certain investigators for the extraction of salicylic acid, because it hydrolyzes readily with the liberation of free acid tending in this way to alter the original acidity of the mixtures and to liberate free salicylic acid on its own account in neutral mixtures. The best solvent was found to be petroleum ether because of its low coefficient of solubility in water and vice versa. Ethyl ether is more soluble in water and serum and vice versa and correspondingly also more troublesome in experiments of this type. A summary of the results that were obtained is presented in table 1.

TABLE 1

*Liberation of free salicylic acid from salicylate (0.05 per cent) in "buffer" solutions and serum and plasma mixtures**

MIXTURE CONTAINING SALICYLATE AND SOLVENTS USED	FREE SALICYLIC ACID IN SOLUTIONS OF DIFFERENT pH VALUES																	
	1.0	2.0	3.0	4.0	5.6	5.9	6.1	6.2	6.4	6.5	6.7	6.9	7.0	7.2	7.4	7.6	8.4	
"Buffers" and ether	+	+	+	+	+	+			+		+		—		—		—	
"Buffers" and chloroform					+			+	+	+	+		—	—	—		—	
"Buffers" and petroleum ether					+			+	+	+	+		—	—	—		—	
"Buffers," 25 per cent beef serum and petroleum ether						—	—		—	—	—	—	—	—	—			
"Buffers," 25 per cent beef plasma and petroleum ether									+	†	+	—	—	—		—		
"Buffers," 25 per cent beef serum and petroleum ether						—	—		—	—	—	—		—	—		—	

* The plus sign (+) means positive test; negative sign (-) = negative test

† Very faint trace.

These results show that the liberation of salicylic acid from salicylate depends on true acidity, i.e., the hydrogen-ion concentration of the solution. The tests for salicylic acid were negative in neutral (pH = 7.0) and alkaline (pH = 7.2 to 8.4) solutions and positive on the acid side (pH = 6.7 to 1.0). The iron test was just about positive at pH = 6.7, and progressively stronger with the increase in acidity, i.e., from pH = 6.5 to 1.0. This is true for the protein free (serum and plasma free) mixtures. However, the mixtures that contained serum and plasma (protein) did not show the presence of free salicylic acid, even at the high degree of acidity of pH = 5.9. Such a degree of acidity of the blood, of course, could never be attained during life. Therefore, it seems improbable that free salicylic acid could be demonstrably liberated from salicylate in the circulation even under the most favorable conditions.

It is interesting to speculate as to the fate of the free salicylic acid liberated in the serum protein mixtures and the possible rôle this might play in body fluids during the treatment of acute rheumatic fever. The degree of acidity of pH = 6.7, at which liberation of the free acid occurs, is very low indeed. Incubations at body temperature were not tried, but it is not believed that the degree of temperature would materially alter the level

of acidity at which liberation occurs. It might be altered quantitatively. It is conceivable that very low degrees of acidity, i.e., in the neighborhood of $\text{pH} = 6.8$ or 6.9 might occur in closed cavities with sluggish circulation as in the articulations, although as far as I know this has not been demonstrated, except for consolidation of the lungs and extracts of these in pneumonia which are claimed by Lord (2) to be slightly acid with a $\text{pH} = 6.0$ to 5.4 . The adequate protein of these regions would play the same rôle as that of the serum and plasma mixtures here reported. That is, protein tends to combine with the free salicylic acid. Bacterial protein probably possesses a similar avidity for the free acid. The rôle of the lipins is conjectural, though perhaps no less important than that of the proteins, since salicylic acid is also lipid soluble. In other words, the possibility of inhibition of bacterial growth in the infected tissues and fluids by virtue of the liberated salicylic acid exists. However it would still be necessary to obtain a high enough concentration of the liberated acid for efficient antiseptic action. It is known that a 0.05 to 0.1 per cent concentration is necessary for this. Since the highest concentration of salicylate that can be obtained in the blood after the administration of the highest therapeutic (3) doses is only about one-fifth of this, or 0.02 per cent, and this is no higher in the joint fluid of arthritis, the rôle of the traces of free salicylic acid that might be liberated even under the most favorable conditions in various regions can be safely dismissed from serious consideration. This will hold even for the circulating blood of fatal asphyxia. We may now proceed to the results of the animal experiments.

LIBERATION OF FREE SALICYLIC ACID FROM SALICYLATE IN THE CIRCULATION

If no free salicylic acid can be demonstrated in the venous blood of the right heart under the most favorable conditions, namely, by changing the reaction of the blood toward acidity through severe and fatal asphyxias, there is no reason to believe that such liberation could occur under ordinary conditions, and

even in rheumatic fever in which the respiratory gaseous interchange is not seriously impaired. This would deny the validity of the theory presented in the fore part of the paper. The results of the experiments to be described sustain this contention, and deny Poulsson's claim.

Observations were made on 5 dogs and 1 cat. The animals were previously morphinized and anesthetized with ether. Blood pressure was recorded from the carotid artery in the usual way. Cannulae were tied into the trachea, a femoral vein and a femoral artery. The salicylate was injected intravenously, using 0.5 to 1.6 cc. of a 10 per cent solution per kilo of body weight, equivalent to about 3 to 8 times the concentration occurring in the blood of patients receiving full therapeutic doses of the drug. Asphyxia was produced by clamping the trachea. Both the venous and arterial bloods were used. Venous blood was obtained from the right ventricle by puncture of the chest at the third to fourth interspace alongside the left sternal margin, using gentle suction on a 10 cc. pipette covered inside with oxalate dust and attached to a suitable needle. The punctures in the right ventricle were always verified at autopsy. Sometimes the cardiac blood was obtained direct by opening the chest after death. Arterial blood was obtained from a femoral artery, containing a cannula attached to a 1 cc. pipette.

The salicylate was injected about fifteen minutes before the bloods were drawn so as to allow time for proper distribution and liberation of the free salicylic acid. The experiments were then conducted in 2 stages, i.e., before and during or after asphyxia. Two sets of blood samples (arterial and venous) were drawn. One set served for determining the change in chemical reaction by the colorimetric method, and the other set was used for the detection of free salicylic acid, by shaking directly with ether and petroleum ether in separatory funnels. 1 cc. of the cardiac (venous) blood was quickly transferred to a celloidin sac containing liquid petrolatum and a little dry oxalate. Arterial blood was collected directly without transferring into celloidin sacs containing oxalate and liquid petrolatum. The

pH of the dialysates was then estimated colorimetrically with phenosulphonephthalein in the usual way.

For the detection of free salicylic acid 10 cc. each of the cardiac venous and arterial bloods were transferred and collected, respectively, into separatory funnels containing a little oxalate and 10 cc. of ether or petroleum ether. These were shaken immediately and continuously for one hour each. Then the ethereal extracts were separated, allowed to evaporate spontaneously and the residues tested for salicyl by treatment with hot water, filtration and application of ferric alum in the same

TABLE 2

*Liberation of salicylic acid from salicylate in the circulation before and during fatal asphyxia**

	WEIGHT OF ANIMAL	TOTAL AMOUNT OF SALICYLATE INJECTED	ESTIMATED CONCENTRA- TION OF SALICYLATE IN THE CIRCULATION	VENOUS BLOOD FROM RIGHT VENTRICLE				ARTERIAL BLOOD FROM FEMORAL ARTERY			
				Before asphyxia		During asphyxia		Before asphyxia		During asphyxia	
				pH	Salicyl	pH	Salicyl	pH	Salicyl	pH	Salicyl
	<i>kgm.</i>	<i>gram</i>	<i>per cent</i>								
Dog	8.5	0.45	0.06	7.3	+	7.3	+	7.3	++	7.3	++
		0.60	0.085	7.3	++	7.1	+	7.2	+	7.0	+
Dog	9.0	0.7	0.086	7.4	—			7.4	—	6.8	—
Cat	2.7	0.2	0.082	7.4	+tr.	7.1	—	7.4	+tr.	7.1	—
Dog	5.7	0.9	0.17	7.5	—	6.9	—	7.5	—	6.9	—
Dog	15.2	1.0	0.073			6.9	—			7.0	—
Dog	7.5	0.5	0.074			6.9	—			7.1	—

* The plus sign (+) means a positive test; the negative sign (—), a negative test.

way as in the experiments in vitro described in the fore part of the paper. It was not always possible to carry out the experiments as uniformly as planned owing to the confusion accompanying the fatal effects of the severe asphyxias induced. In some experiments the bloods were obtained just as the animal was dying or immediately after death. The results obtained are summarized in table 2.

The results appear somewhat irregular. This is due to the use of ordinary ether as the immiscible solvent in the first 3 experiments (dog 8.5, dog 9.0 and cat 2.7 kilos). Positive

tests for salicyl in some of these experiments were obtained with both sets of bloods, i.e., before and during asphyxia, and when these were alkaline. This simply means that there was some contamination of the ethereal extract with salicylate from the blood because of its solubility in the ether. These experiments are purposely included to indicate a source of error which might lead to an erroneous conclusion. In the remaining experiments these difficulties were avoided by using petroleum ether as the immiscible solvent with the result that the salicyl tests were uniformly negative.

It is seen that the severe asphyxias markedly reduced the alkalinity of the bloods. In 4 animals the asphyxial blood was very faintly acid ($\text{pH} = 6.8$ and 6.9).

However, despite this favorable change in reaction for the liberation of free salicylic acid, the tests were entirely negative, and do not sustain Poulsson's claim. This confirms fully the experiments with the mixtures of salicylate and serum and plasma *in vitro*. The degree of acidity at which liberation of free salicylic acid occurs in aqueous solutions is unattainable even at death from asphyxia. Obviously this is also unattainable during life. It is, therefore, justifiable to conclude that the theory of the absence of a right-sided valvular endocarditis in rheumatic fever as due to the antiseptic qualities of the free salicylic acid liberated by virtue of the greater carbon dioxide content of the venous blood is untenable. The explanation of this peculiar and interesting phenomenon must be sought elsewhere.

The results of the experiments reported in this paper furnish the basis for an additional deduction, namely, that fluids of joints and similarly enclosed regions would need to be more highly acid and freer from protein and other constituents than is probably the case in order to contain free salicylic acid and explain the therapeutic relief from salicylate medication according to the antiseptic theory. This is fully confirmed by the negative results of a previous publication (3) which reported observations on the direct ethereal extraction of joint fluids

from patients suffering with rheumatic fever and receiving salicylate.

CONCLUSIONS

1. Free salicylic acid is demonstrably liberated from sodium salicylate at a very low degree of acidity, namely, an acidity whose hydrogen-ion concentration corresponds to $\text{pH} = 6.7$; more definitely at $\text{pH} = 6.5$.

2. The presence of 25 per cent serum or plasma in salicylate "buffer" mixtures prevents the liberation of free salicylic acid at the high degree of acidity of $\text{pH} = 5.9$.

3. Therefore, it is improbable that free salicylic acid could be demonstrated in the circulation during life.

4. This was fully confirmed on animals subjected to fatal asphyxia and whose cardiac and arterial bloods were rendered very slightly acid ($\text{pH} = 6.8$ or 6.9).

5. Consequently, the theory that free salicylic acid, liberated by virtue of the greater CO_2 content of venous blood of the right heart, exerts an antiseptic action and prevents the development of a right-sided auriculo-ventricular (tricuspid) endocarditis in rheumatic fever is untenable. An explanation of this phenomenon must be sought elsewhere.

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EPINEPHRINE HYPERGLYCEMIA. I

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Epinephrine as a glycogenolytic agent has for several years been extensively employed in physiological investigations. Intensively it has been used chiefly as a means to reduce or eliminate entirely hepatic glycogen when used in conjunction with phloridzin (1), (2). Concerning the mode of action of epinephrine itself upon the hepatic mobilization of sugar there exists considerable obscurity, notwithstanding the more or less current opinion that it is an asphyxial process. The asphyxial idea has had chief support from the work of Ritzmann (3) who observed a parallelism between pressor action and sugar mobilization under the influence of continuous intravenous infusions of weak epinephrine solutions. Sansum and Woodyatt also suggestively support this same idea without making any special study of this question in particular but incidentally parallel the action of epinephrine with anesthetics in general which they have convincingly shown to produce a sugar mobilization by a common mechanism and this appeared to be asphyxia.

MacLeod (4) reported hyperglycemia to follow alternately obstructing and releasing the portal vein, while thrombosis of the portal vein in man has never been observed to give rise to hyperglycemia, and furthermore ligation of hepatic arteries and also Eck's fistulae were without effect. These facts are interpreted by MacLeod to mean that hepatic artery occlusion does not lead to a sufficient degree of asphyxia to effect sugar liberation and that in clinical portal thrombosis there is not enough blood passing through the liver to wash out the liberated dextrose, whereas alternately completely occluding and releasing the portal flow both sufficiently asphyxiates to effect glycogen-

olysis and subsequently washes out liberated dextrose. This author then having proved the existence of a localized asphyxial origin of glycogenolysis is prone to parallel the effects of sympathetic nerve stimulation to localized asphyxia. However he failed to find any consistent glycogenolysis in partial portal vein occlusion and furthermore found that in the bloodless liver the glycogenolysis appeared to be more rapid in lobes whose sympathetic nerves were stimulated than in unstimulated control lobes which would seem to indicate a contributory specific glycogenolytic nervous mechanism.

That epinephrine acts upon the liver through sympathetic nerves is generally accepted from the general sympathomimetic action of epinephrine and that ergotoxin which prevents epinephrine action on myoneural junctions (Dale (5)) also prevents or diminishes hyperglycemia (Miculicich (6)). This might be explained on the basis of preventing vasoconstriction in the blood vessels of the liver and hence preventing stasis and localized acidosis. This view necessitates the assumption that hepatic blood vessels are far more susceptible to epinephrine than other vessels of the body since in order to produce hyperglycemia the best practical means are those in which epinephrine is so slowly absorbed into the blood stream as to be essentially without effect on total blood pressure. These means are subcutaneous or intraperitoneal injections or slow infusions into the blood stream directly (7), (8), (9). The inconsistency here appears to lie in the fact that subcutaneous injections of epinephrine may produce no change in total blood pressure yet at the same time are powerful stimulants to glycogenolysis, and furthermore intravenous injections which can safely be used to demonstrate acute pressure changes are too transient or in some other manner incapable of producing glycogenolysis. Underhill claims that dose for dose subcutaneous administration is more efficient as a glycogenolytic agent than when given by slow intravenous infusions (7).

McGuigan and Mostrom (10) hold that epinephrine produces vascular constriction by virtue of some special atomic groups in the molecule while other atomic groups effect glycogenolysis.

This conclusion is based on their observations that on partial oxidation the glycogenolytic action is destroyed before the pressor action. The validity of this view is to our minds more or less annulled by our own heretofore unpublished observations that formaldehyde which destroys the pressor action (Cramer (11), Budai (12)) also destroys the glycogenolytic action of epinephrine. Thus the tying up of the secondary amine group of the molecule with formaldehyde (methylene radicle) suffices to eliminate both types of action. Obviously there is still the possibility that changes in size or character of the molecule prevents its activity altogether even though different groups may conceivably be involved in glycogenolysis and pressure action respectively.

We furthermore recognize that changes in distribution of the blood follow the use of epinephrine as a drug which means that while certain vascular areas contract others dilate possibly leaving the carotid pressure quite unchanged.

In view of all these facts it appeared to us to be feasible to view this problem from another angle. If epinephrine hyperglycemia (hyperglycemia in the sense of an increase in sugar from any chosen base line) is due primarily to acidosis of a general or localized character such should be observable from a study of blood neutrality. Peters and Geylin (13) indeed have reported a fall in alkaline reserve capacity after subcutaneous injections of epinephrine coincident to the usual rise in blood sugar concentration. These authors are of the opinion that the acidosis preceded and was the causative mechanism of epinephrine hyperglycemia. We also have repeatedly confirmed this apparent parallelism of hyperglycemia and acidosis in rabbits in this laboratory. However, MacLeod and Fulk (14) have shown that injections of large amounts of sugar alone will produce an increase in hydrogen ion concentration in blood, consequently it should be questioned whether the fall in reserve after epinephrine might not be secondary to the rise in sugar rather than that the rise in sugar is secondary to fall in reserve. The latter is certainly true in certain conditions in which acidosis

is primarily induced (15). But such a parallelism does not prove the action of epinephrine to be in this manner.

EPINEPHRINE GLYCOGENOLYSIS IN PHLORIDZIN DIABETES

If the acidosis is the primary factor, primary to stasis of blood or anoxemia in the liver, then in such circumstances as low blood sugar concentration or absence of hepatic glycogen the epinephrine acidosis should still be in evidence without the development of a high degree of hyperglycemia.

This line of argument appeared to us worthy of investigation with the likelihood of throwing some light on the mechanism of glycogenolysis from epinephrine.

To obtain conditions adequate to prevent a rise of blood sugar of serious grade the well known condition of phloridzin diabetes was utilized. Rabbits were used for the most part in this phloridzin study, though one confirmatory experiment was made on the dog to prove to our own satisfaction that the results obtained were not specific to the rabbit. Obviously anesthetics were avoided entirely.. Blood for analysis or blood sugar and alkaline reserve capacity (by methods previously described (16)) was taken from the ear veins of rabbits and the saphenous vein from the dog. After phloridzin in large amounts and for a more or less extended period of administration there was observed a fall in blood sugar and subsequently and roughly paralleling the fall in sugar a fall in alkaline reserve capacity of whole blood. This was invariably observed (table 1). Glycosuria appeared long before it was possible to induce a condition of marked hypoglycemia. In fact it is rather difficult to induce the condition of hypoglycemia in the rabbit, and very heavy phloridzin administration for quite a period is usually required. Yet Sansum and Woodyatt have shown, as well as has Lusk, that phloridzin alone does not completely exhaust the glycogen supply of the liver.

With the condition of pre-existing hypoglycemia a blood reservoir is established into which dextrose from the liver can be poured with little danger of over-reaching the normal limits

of blood sugar. Epinephrine injected under these conditions should (a) either further lower the alkaline reserve by virtue of hepatic asphyxia with increase in sugar, or (b) increase the sugar with a rise or no change in reserve. The latter practically uniformly obtained in our series (tables 2, and 3). It may be argued that localized hepatic acidosis may be counter-balanced or obscured by the relative alkalosis in the general circulation sub-

TABLE 1
Effect of phloridzin on alkaline reserve capacity of whole blood and blood sugar concentration in the rabbit

ANIMAL NUMBER	WEIGHT	TIME	PHLORID- ZIN INJECTED	HEMOR- RHAGE	BLOOD SUGAR PER CUBIC CENTI- METER	ALKALINE RESERVE CAPACITY
	<i>kgm.</i>	<i>1920</i>	<i>gram</i>	<i>cc.</i>	<i>mgm.</i>	
1	1.5	July 11, 10:30 a.m.	0.3	2		0.62
		11:30 a.m.		4	1.37	0.61
		2:20 p.m.				
		3:20 p.m.	0.3	4	0.71	0.56
		4:30 p.m.		4	0.53	0.56
		5:10 p.m.				
		5:50 p.m.		4	0.35	0.52
2	2.0	July 22, 10:15 a.m.	0.5			
		10:45 a.m.	0.5	4	0.78	0.51
		11:30 a.m.		4	0.78	0.50
		1:30 p.m.				
		2:15 p.m.	0.5	4	0.75	0.57
		3:40 p.m.		4	0.75	0.45
		5:00 p.m.				
		6:00 a.m.		4	0.63	0.41
		7:00 p.m.		4	0.61	0.39
		July 23, 9:30 a.m.		4	0.98	0.48

sequent to the rise in sugar values. Yet according to Stiles and Lusk (17), in complete phloridzin diabetes sugar is not utilized unless present in very large amounts. We also find that in such instances as we could be sure of as not recovering from phloridzin effects subcutaneous injections of dextrose did not change the alkaline reserve values of blood. If, however, phloridzination was relatively light, there did occur after epinephrine

TABLE 2
Effect of epinephrine injections on phloridzinized rabbit

ANIMAL NUMBER	WEIGHT	TIME	PHLORID- ZIN INJECTED	HEMOR- RHAGE	BLOOD SUGAR PER CUBIC CENTI- METER	ALKA- LINE RE- SERVE CAPACITY	ADRE- NALIN*
	<i>kgm.</i>	<i>1920</i>	<i>gram</i>	<i>cc.</i>	<i>mgm.</i>		<i>cc.</i>
3	3.0	August 5, 1:50 p.m.	0.7				
		3:30 p.m.	0.7				
		5:10 p.m.	0.7				
		7:30 p.m.		8	1.13	0.56	
		August 6, 8:45 a.m.	0.8				
		10:30 a.m.		4	1.07	0.54	
		10:45 a.m.	0.8				
		2:00 p.m.		4	0.84	0.55	
		2:30 p.m.	0.8				
		5:00 p.m.		4	0.93	0.41	
		5:25 p.m.					0.3
		6:30 p.m.		4	1.51	0.38	
		7:45 p.m.		4	2.06	0.50	
		8:45 p.m.		4	1.88	0.50	
		August 7, 8:00 a.m.	0.8				
		August 8, 10:30 a.m.	0.8				
4	3.0	3:45 p.m.	0.8				
		5:00 p.m.		4	0.80	0.40	
		5:20 p.m.					0.3
		7:20 p.m.		4	1.07	0.40	
		8:20 p.m.					0.5
		8:30 p.m.	0.8				
		9:20 p.m.		4	2.05	0.40	
		August 9, 8:00 a.m.	0.8				
5	3.0	5:00 p.m.	0.8				
		August 10, 8:00 a.m.	0.8				
		12:00 m.	0.8				
		5:00 p.m.	0.8				
		August 11, 10:00 a.m.	0.8				
		2:00 p.m.	0.8				
		5:00 p.m.	0.8				
		7:15 p.m.		4	0.75	0.42	
		7:35 p.m.					0.4
		8:35 p.m.		4	0.84	0.42	
		9:35 p.m.		4	1.13	0.40	
6	2.8	August 12, 9:55 a.m.	0.8				
		3:30 p.m.	0.5				
		6:20 p.m.	0.8				
		7:20 p.m.		4	0.65	0.44	
		7:50 p.m.					0.4
		9:00 p.m.		4	0.98	0.44	

* Adrenalin chloride, P. D. & Co., 1:1000, was used throughout, injected subcutaneously.

TABLE 3

Dog, weight 12 kgm., fasting but supplied with water. Dog was given for three successive days two separate subcutaneous injections of 1 gram phloridzin

TIME	PHLORIDZIN INJECTED	HEMOR- RHAGE	BLOOD SUGAR PER CUBIC CENTIMETER	ALKALINE RESERVE CAPACITY	ADRENALIN
1920	gram	cc.	mgm.		cc.
August 21, 10:30 a.m.	1				
12:10 p.m.	1				
2:30 p.m.	1				
4:25 p.m.	1				
5:10 p.m.		4.5	0.46	0.42	
6:10 p.m.					0.5
7:30 p.m.		4.0	0.55	0.43	

as well as after dextrose injections a rise in reserve within two to three hours, secondary apparently to either wearing off of the effects of phloridzin or utilization of the blood sugar or more probably both.

Bearing also on the mode of epinephrine action, but evidently differently interpreted by the authors themselves, Sansum and Woodyatt report in experiment VIII of their series, no essential change in "acetone bodies" during or following epinephrine periods. This appears to indicate the absence of any definite causative acidosis.

It may also be argued that epinephrine after phloridzination prevents a fall of reserve by the same mechanism MacLeod suggests relative to the absence of hyperglycemia following Eck's fistula or portal vein thrombosis, namely, stasis of such a degree that the acid bodies are not washed out of the liver. Yet with normal animals epinephrine liberates great quantities of sugar and also directly or indirectly lowers the alkaline reserve. In phloridzination it would then have to be assumed that portal stasis was too complete to wash out acid bodies yet incomplete enough to wash out sugar.

This problem might have been further elucidated by a chemical examination of portal versus hepatic vein blood, but we have not as yet considered this feasible on account of the relative intolerance of phloridzinized animals to general anesthesia and

the complications of anesthesia with traumatic effects incident to a serious laparotomy.

CIRCULATORY EVIDENCES REGARDING EPINEPHRINE HYPER-GLYCEMIA

According to the idea of Ritzmann, epinephrine produces hyperglycemia subsequent to and dependent upon hepatic or portal vasoconstriction producing thereby a degree of portal stasis adequate to cause anoxemia or local hepatic asphyxia. MacLeod also believed that such a stasis is either causative or contributory.

If such a stasis exists after subcutaneous injections of epinephrine it should then cause a backing up of blood in the portal system which in turn would of necessity cause an increase in portal vein pressure.

To test this possibility we have connected the splenic vein of etherized dogs to a water manometer in such a way as to record portal pressure as reflected in the splenic tributary. In some experiments the hepatic artery was ligated to simplify the liver circulation. Subcutaneous injections of epinephrine in 1 to 4 mgm. doses failed to produce any change whatsoever in the splenic vein pressure. Subsequent injections of epinephrine into the femoral vein promptly produced a marked rise of venous pressure in the portal system.

These experiments, then, would strongly indicate the absence of vasoconstriction in the hepatic portal vein ramifications, at any rate enough to affect measurably the venous pressure. Bearing in mind the fact of the enormous volume of portal blood flow one would think that a portal stasis of considerable degree would certainly be necessary to produce hepatic asphyxia. MacLeod, indeed, found partial portal obstruction as well as ligation of hepatic arteries without effect in producing glycogenolysis. Those experiments in which he alternately completely obstructed and released the portal vein could hardly be considered fair evidence of the asphyxial origin of glycogenolysis by epinephrine since even an excised and autolyzing liver loses its glycogen;

consequently it appears that such an experiment more nearly approaches excision or post-mortem glycogenolysis than a physiological process.

It may possibly be argued that epinephrine simultaneously constricts intestinal vessels so as to retard the inflow of venous blood to the portal system. This would necessitate either an increase in systemic arterial pressure or a diminution of cardiac action. On the other hand it has been abundantly proved that minimal effective doses of epinephrine intravascularly injected relax some arterioles rather than constrict them, which would prevent a systemic rise of pressure.

Since it is easily possible to choose a dose of epinephrine which, subcutaneously injected produces hyperglycemia without change in either systemic or portal blood pressure, and since neither hepatic ligation nor partial portal obstruction produces hyperglycemia, it appears to us that these evidences alone would necessitate abandonment of the anoxemia or asphyxia theory of epinephrine glycogenolysis. The parallelism between glycogenolysis and pressure changes recorded by Ritzmann may be satisfactorily considered, as a working hypothesis, to be merely instances of parallelism and not cause and effect.

THE EFFECTS OF ACIDS ON CHANGES IN BLOOD SUGAR AND ALKALINE RESERVE CAPACITY OF BLOOD FROM EPINEPHRINE INJECTIONS

If one considers epinephrine hyperglycemia as a consequence of localized hepatic asphyxia by blood stasis, such an acidosis can become effective intracellularly after exhaustion or neutralization of alkaline reserve materials within the cells which in turn should draw upon the capillary blood reserve. If this be the case it appears reasonable to assume that by a preliminary exhaustion of blood reserves acidosis would be more readily induced and with a far greater relative glycogenolysis. Yet we remember that in general acids in themselves relax blood vessels which should counteract to some extent constriction stasis.

To put this question to the test varying degrees of acidosis were induced by either potassium dihydrogen phosphate or hydrochloric acid by stomach. These means lower quite readily the alkaline reserve of blood, and being absorbed into or influencing first the portal blood would thus be equivalent to injection into the portal system.

After acid administration, epinephrine subcutaneously injected produced on an average slightly more reducing substances in blood than in the control series, though in some instances previous acidification rendered epinephrine less effective than the average of nonacidified instances. Nevertheless, granting the existence of a slightly increased potency of epinephrine in the acid series such a slightly increased potency is far from being of an order comparable to the total effective glycogenolytic action of epinephrine itself.

By forcing the acid administration till a sufficiently low value of alkaline reserve capacity was obtained, it was found that there occurred the usual epinephrine hyperglycemia with no characteristic or significant fall in reserve. This is quite like the results obtained with acidosis induced by phloridzin. There was in the hydrochloric acid acidosis, however, no serious depletion of hepatic glycogen hence the usual glycogenolytic response after epinephrine. More acid by stomach still further lowered the reserve capacity without any significant change in sugar values. Thus it appears to us that the apparent contradictory evidence given above loses its force in view of the fact that when the peripheral blood acidity has been so increased by hydrochloric acid as to be as high or higher than that produced by epinephrine alone then if further acid is given to further lower the reserve the glycogenolysis is negligible in comparison to the glycogenolysis effected by epinephrine acting without any subsequent change in reserve whatsoever.

If acidosis is considered causative in epinephrine hyperglycemia the question arises whether for a given fall of reserve by hydrochloric acid and by epinephrine there exists any comparable glycogenolysis (tables 4, 5 and 6).

TABLE 4

*Effect of epinephrine on blood sugar and alkaline reserve capacity of whole blood.
Control series. Rabbit*

ANIMAL NUMBER	WEIGHT	TIME	HEMOR- RHAGE	BLOOD SUGAR PER CUBIC CENTI- METER	ALKALINE RESERVE CAPACITY	ADRE- NALIN
	<i>kgm.</i>	<i>1920</i>	<i>cc.</i>	<i>mgm.</i>		<i>cc.</i>
7	2.1	November 16, 4:00 p.m. 5:05 p.m.	3	0.87 2.26		0.5
8	2.0	November 17, 4:00 p.m. 4:05 p.m. 5:05 p.m.	3 3	0.75 2.26		0.5
9	2.4	November 18, 10:40 a.m. 10:43 a.m. 11:43 a.m.	3 3	0.84 2.26		0.5
10	2.0	November 18, 2:30 p.m. 3:00 p.m. 3:36 p.m. 4:36 p.m. November 19, 10:00 a.m. 10:20 a.m. 11:20 a.m.	5 3 5 5 5 5	0.98 2.51 0.94 2.51	0.72 0.55 0.62 0.55	0.5 0.5 0.5
11	2.0	November 19, 4:45 p.m. 4:55 p.m. 5:55 p.m. November 20, 10:53 p.m. 10:55 p.m. 11:55 p.m.	5 5 5 5 5	1.19 2.83 0.84 1.74	0.66 0.50 0.60 0.56	0.5 0.5 0.5
12	2.2	November 26, 2:30 p.m. 2:45 p.m. 3:45 p.m. November 27, 3:30 p.m. 3:46 p.m. 4:36 p.m. November 28, 11:20 a.m. 11:57 a.m. 12:57 p.m. November 30, 10:00 a.m. 10:20 a.m. 11:20 a.m.	5 5 5 5 5 5 5 5 5 5 5	0.94 2.83 0.94 2.51 0.94 0.98 2.06	0.60 0.56 0.64 0.63 0.63 0.60 0.63	0.5 0.5 0.5 0.5 0.5 0.5

TABLE 5

*Effect of 1 per cent HCl on epinephrine hyperglycemia and alkaline reserve status.
Acid administered by stomach tube. Rabbit*

ANIMAL NUMBER	WEIGHT	TIME	HEMOR- RHAGE	HCl 1 PER CENT	BLOOD SUGAR PER CUBIC CENTI- METER	ALKA- LINE RESERVE	ADRE- NALIN		
	<i>kgm.</i>	<i>1920</i>	<i>cc.</i>	<i>cc.</i>	<i>mgm.</i>		<i>cc.</i>		
13	2.4	{	December 23, 11:30 a.m.	2	65		0.68		
			11:50 a.m.						
			2:00 p.m.	2	20		0.45		
			2:20 p.m.						
			3:30 p.m.	2			0.40		
			4:30 p.m.	2			0.39		
			5:20 p.m.	2			0.35		
			6:00 p.m.	2			0.36		
14	2.1	{	December 24, 9:27 a.m.		60				
			11:45 a.m.	2			0.43		
			2:15 p.m.	2			0.42		
			3:30 p.m.	2			0.46		
15	3.0	{	December 1, 2:45 p.m.	5	25	0.85	0.64		
			3:00 p.m.						
			4:00 p.m.	5	18	0.94	0.57		
			4:30 p.m.						
			5:30 p.m.	5		0.98	0.54		
16	2.0	{	December 2, 10:00 a.m.	5	25	1.13	0.69	0.5	
			10:40 a.m.						
			11:40 a.m.	5		1.25	0.53		
			12:00 m.						
			1:00 p.m.	5		3.23	0.44		
17	2.0	{	December 2, 3:00 p.m.	5	30	1.08	0.50	0.5	
			3:35 p.m.						
			4:38 p.m.	5		1.10	0.40		
			4:58 p.m.						
			6:00 p.m.	5		2.83	0.39		
18	2.0	{	December 17, 11:30 a.m.		50				
			1:00 p.m.	4			0.90		
			1:30 p.m.		25				
			2:40 p.m.	4			0.99		
			3:00 p.m.						
			4:00 p.m.	4			2.51		
			4:32 p.m.						
			5:30 p.m.	4			3.76		

TABLE 5—*Continued*

ANIMAL NUMBER	WEIGHT	TIME	HEMOR- RHAGE	HCl 1 PER CENT	BLOOD SUGAR PER CUBIC CENTI- METER	ALKA- LINE RESERVE	ADRE- NALIN
	<i>kgm.</i>	<i>1920</i>	<i>cc.</i>	<i>cc.</i>	<i>mgm.</i>		<i>cc.</i>
19	2.3	December 20, 11:00 a.m.	4		1.03	0.58	
		11:30 a.m.		60			
		12:40 p.m.	4		0.98	0.44	
		1:05 p.m.		15			
		2:00 p.m.	4		1.03	0.40	
		2:15 p.m.					0.5
		3:25 p.m.	4		3.75	0.37	
		3:40 p.m.					0.5
		5:00 p.m.	4		3.75	0.42	
20	2.2	December 21, 2:30 p.m.		50			
		3:30 p.m.	2			0.46	
		4:00 p.m.		25			
		5:00 p.m.	4		0.98	0.44	
		5:15 p.m.		15			
		6:15 p.m.	4		0.98	0.42	
		6:30 p.m.					0.5
		7:30 p.m.	4		1.25	0.40	
21	1.9	December 22, 9:50 a.m.		50			
		10:50 a.m.	2			0.48	
		11:13 a.m.		25			
		12:13 p.m.	2			0.44	
		1:50 p.m.	2			0.40	
		2:30 p.m.	2			0.36	
		2:55 p.m.					0.5
		3:25 p.m.	2			0.40	
		4:00 p.m.	2			0.40	
		4:30 p.m.	2			0.44	

In the table may be seen data bearing on this point. It is seen from the table that hydrochloric acid is a powerful depressor of the reserve yet is incomparably weaker as a glycogenolytic agent than epinephrine. It may be argued as suggested in the fore part of this paper that epinephrine acidosis is a strictly localized affair and is not adequately measured by its influence on circulating blood in general. Yet alimentary hydrochloric acid is also essentially hepatic since it first must pass through the

TABLE 6
Effect of KH_2PO_4 on epinephrine hyperglycemia in the rabbit

ANIMAL NUMBER	WEIGHT	TIME	HEMOR- RHAGE	KH_2PO_4	BLOOD SUGAR PER CUBIC CENTI- METER	ADRE- NALIN
	<i>kgm.</i>	<i>1920</i>	<i>cc.</i>	<i>gram</i>	<i>mgm.</i>	<i>cc.</i>
22	2.4	{ November 13, 10:40 a.m. 2:10 p.m. 3:10 p.m.	3 3	0.8	1.03 2.82	0.5
23	2.1	{ November 15, 11:00 a.m. 2:30 p.m. 3:30 p.m.	3 3	0.8	0.98 2.05	0.5
24	2.0	{ November 16, 11:00 a.m. 2:00 p.m. 3:05 p.m.	3 3	0.8	1.41 3.28	0.5

liver before reaching the peripheral circulation and consequently the same reasoning may be applied to this form of acidosis. In hydrochloric acid acidosis, the acidosis is primarily extrinsic to the cell while anoxemia would give rise to an intrinsic development of acid. There is however no comparable glycogenolysis produced by hydrochloric acid even when the fall in reserve is far greater than that produced by epinephrine.

On comparing the amount of fall of reserve and the amount of hyperglycemia induced by epinephrine in different animals and in the same animals on successive days (table 4) there is found a marked fluctuation in reserve depression, greater in degree than that in hyperglycemia production. There was no consistent parallelism that could be depended on for a criterion of mutual interdependence.

IS THE FALL IN RESERVE AFTER EPINEPHRINE DUE TO OVER- ACTIVITY OF THE RESPIRATORY CENTER?

It has been shown that epinephrine increases pulmonary ventilation (18), which theoretically might be expected to be a causative factor in the fall of reserve. This possibility was studied. Rabbits were urethanized (1 gram per kilo) by stom-

ach, then artificial respiration established, and in one instance bilateral pneumothorax was produced by rib resection. After a time control specimens of blood were drawn from the jugular vein and analyzed for reserve capacity. Then epinephrine in usual dosage was given subcutaneously. After one-half to one hour blood was again taken. Reserve values fell following epinephrine medication precisely like those under normal conditions of respiration.

It is theoretically possible, though we believe quite improbable, that an accelerated blood flow through the lungs might in these instances lower reserve values even though lung movements be maintained constant.

These experiments we believe essentially eliminate the respiratory center from responsibility for the lowering of alkaline reserve capacity.

MORPHINE AND EPINEPHRINE

Morphine, as is well known causes a depression of the respiratory center with a consequent rise in alkaline reserve capacity of blood.

It seemed profitable to see whether morphine, by raising the reserve capacity of blood, has any depression or augmentation effect on epinephrine hyperglycemia and reserve capacity (table 7).

It is to be seen from the table that occasionally the reserve rise by morphine is just counteracted by the reserve fall by epinephrine, while for the most part the depression of reserve by epinephrine greatly preponderates. In sugar titre, there was obtained the usual rise, while the depression of reserve appeared to be more closely related to the total amount of reserve, i.e., the greater the reserve value, the greater the fall under epinephrine, than to sugar production.

DOES HYPERGLYCEMIA PER SE CAUSE A FALL IN RESERVE?

In view of the experiments with phloridzin and with hydrochloric acid, as well as the rather constant fall of reserve of some degree from epinephrine, the question of relationship of excess

TABLE 7

The effect of morphine on epinephrine hyperglycemia and alkaline reserve status

ANIMAL NUMBER	WEIGHT	TIME	HEMOR- RHAGE	MOR- PHINE	BLOOD SUGAR PER CUBIC CENTI- METER	ALKA- LINE RESERVE	ADRE- NALIN
	<i>kgm.</i>	<i>1920</i>	<i>cc.</i>	<i>mgm.</i>	<i>mgm.</i>		<i>cc</i>
25	2.0	December 4, 10:15 a.m.		1.0			
		10:40 a.m.		1.0			
		11:40 a.m.	5		1.03	0.64	
		12:02 p.m.					0.5
		1:10 p.m.	5		2.83	0.63	
26	2.0	December 4, 2:00 p.m.		3.0			
		3:00 p.m.	5		0.93	0.66	
		3:24 p.m.					0.5
		4:24 p.m.	5		1.26	0.60	
		5:20 p.m.		10.0			
27	2.2	5:50 p.m.	5		1.26	0.60	
		December 5, 10:20 a.m.		10.0			
		11:20 a.m.	5		1.13	0.66	
		11:42 a.m.					0.5
		12:42 p.m.	5		3.23	0.65	
28	2.0	December 7, 10:35 a.m.		10.0			
		11:15 a.m.	2			0.74	
		12:15 p.m.	2			0.77	
		1:45 p.m.	2			0.80	
		3:20 p.m.	2			0.79	
29	2.00	3:39 p.m.					0.5
		4:40 p.m.	2			0.64	
		December 7, 4:00 p.m.		17.6			
		4:15 p.m.	2			0.65	
		5:15 p.m.	2			0.72	
		5:35 p.m.					0.5
		6:10 p.m.	2			0.67	

sugar to reserve values required consideration. In a previous paper we have reported the effects of subcutaneous injections of dextrose in such amounts as to cause a distinct hyperglycemia (16). It was found that there were no significant changes in reserve. Consequently the excess sugar cannot be held responsible for reserve changes.

GENERAL DISCUSSION

All in all, the results of the experiments given above appear irreconcilable from the standpoint of acidosis being the causative mechanism of epinephrine glycogenolysis.

Epinephrine causes, on subcutaneous injections in adequate amounts, a fall in alkaline reserve capacity in normal animals. The conclusions from this fact would point to acidosis as causative. This is supported by the failure of hyperglycemia from dextrose injections to cause a considerable fall in reserve. On the other hand when the alkaline reserve is greatly lowered by other means, such as in acidosis from phloridzin or by gastric administration of strong acids epinephrine still exerts its usual glycogenolytic activity without further change in alkaline reserve capacity. That the drug is acting is proven by the hyperglycemia induced. If acidosis within the liver is causative, and is induced by hepatic vasoconstriction such that a very marked fall of reserve occurs in normal animals it would be expected that an equivalent acid production should occur when acidosis pre-exists. It may be argued that the low level of reserve produces a condition of hepatic lability that undetectable changes in acidosis are effective. Yet such a change in lability is not indicated by any marked or consistent increased glycogenolytic potency of epinephrine in animals whose reserve of alkali has been partially exhausted by acid administration. Furthermore for a given fall of reserve by acid by stomach and "acid" from epinephrine, epinephrine is incomparably the more powerful glycogenolytic agent.

McDanell and Underhill (19) report that alkalies decrease slightly the efficiency of epinephrine as a glycogenolytic agent. However they observed that this depression of glycogenolytic power was greater with large doses of epinephrine than with small doses; the opposite to what one would be logically led to suspect. Alkalies did not, however, abolish nor very greatly lower epinephrine hyperglycemia.

We have been unable to demonstrate any portal stasis as reflected in changes in pressure, following subcutaneous injec-

tions of epinephrine in etherized dogs. This may be taken to mean the absence of any high degree of stasis of portal blood in the liver. The same negative results are obtained after previous ligation of the hepatic artery.

If epinephrine produces acid by vasoconstriction, or acid by any other means, causal to the production of glycogenolysis it would be a unique mechanism in that the amount of detectible acid produced is nil in conditions of a fairly high degree of acidosis while in normal conditions considerable acid production does occur. There is produced essentially the same quantitative glycogenolysis, as measured by hyperglycemia, regardless of whether or not acidosis pre-exists. It would mean that acid production (vasoconstriction) depends upon the amount of buffer alkalies to be neutralized; yet artificially changing the buffer reserves has no significant effect upon the amount of glycogenolysis produced by epinephrine. In this connection, it may be stated that we have made no control study of the remote possibility of variations in renal activity determining the state of acid-base balance in the conditions outlined above. This possibility seems entirely unlikely since acids by stomach readily depress the blood reserves while acidity by epinephrine becomes more or less self-limited.

All of the available data therefore do not form together a consistent ensemble picture of the mode of epinephrine hyperglycemia production. They rather point to independent processes, one having to do with glycogenolysis, the other with production of acid, or its counterpart, the disappearance of buffer alkalies.

CONCLUSION

From a study of all available evidences in the literature together with the data presented in this paper we are forced to conclude that epinephrine glycogenolysis cannot be satisfactorily explained on the basis of hepatic asphyxia or acidosis. The real mechanism of epinephrine mobilization of carbohydrates therefore is as yet undetermined.

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THE EFFECT OF BENZYL BENZOATE ON THE LEUCOCYTES OF THE RABBIT

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While treating spasmodic dysmenorrhea with benzyl benzoate one of us observed in two patients the recrudescence of an old appendicitis not accompanied by the usual increase in white blood cells. Later a similar experience was had by another member of this department. The three respective attacks developed after a total dose of 240, 300 and 120 minims of the actual substance given over a period of eight days, ten days and five days respectively. In the same order, the white blood cells were found to be 5000, 4000 and 6000 in number at the height of the attack. Our curiosity was aroused at once by the low blood count and the coincidence of the similarity of the clinical picture occurring during benzyl benzoate administration. We turned to the literature for information on the influence of benzyl benzoate on the blood picture in general and found none. (The work of Heller and Steinfield appeared only after the completion of our experimental study.) We, then turned to the literature on substances related to benzyl benzoate. There, we found a great deal of information on the influence of benzol on the blood and some meager reports on blood studies relative to benzoic acid and sodium benzoate. Briefly summing up these facts we learned that:

Benzol is a leucotoxin (Selling); it produces a diphasic polymorphonuclear leucopenia in rabbits which is not influenced by splenectomy (Weiskotten et al.); it depresses the anti-infectious powers of the body (Rusk, Hektoen); and it allows quiescent or

latent infections of rabbits to become acute, then changing the leucopenia into a leucocytosis abolishing at the same time the diphasic character of the benzol curve (Weiskotten et altri). Benzol is in part eliminated in the urine as phenol (Brewer and Weiskotten). Benzoic acid and the benzoates in general are not as well known in their biological behavior. It is well known that their urinary end product is hippuric acid instead of phenol. Their influence on the blood picture has been investigated only superficially but it is known that, in man, benzoic acid and sodium benzoate tend to produce a slight leucocytosis (Wiley). Since these blood studies were carried on over a short period only the end phenomena are not known.

In the light of these facts our clinical observations suggested to us that benzyl benzoate under certain conditions might act somewhat like benzol in so far as it may have depressed the anti-infectious powers of the body sufficiently to allow a chronic infection to return to acute states as manifested by the low white blood count found at the height of the recrudescence of a chronic inflammation. We refrain from making a lengthy theoretical explanation of our assumption. Contrary to what is known about the benzoates we assumed that since benzyl benzoate has two benzene rings, one of which is loosely attached, it might be possible that in the breaking down one benzene structure could be set free, while the remaining portion of the structure experiences the usual conversion into hippuric acid. Since the benzene nucleus of aromatic substances is extremely stable on account of its resistance to oxidation (Holland and May), sufficient quantities of benzyl benzoate whose accumulative action we do not know, might therefore produce leucotoxic changes.

In order to clear up this question we decided to carry out several groups of experiments, the first series of which we report here and which contains only data on blood pictures in rabbits to whom benzyl benzoate had been administered.

TECHNICAL POINTS

Comparatively large doses of benzyl benzoate can be administered to the rabbit by mouth or hypodermically without any

apparent ill effects. In Macht's experiments, 1 cc. per kilo weight of the substance did not disturb this animal. We found later that even larger doses will not produce any more toxic symptoms than lassitude, loss of appetite and loss of weight. But it does not follow that man is equally resistant because it is well known that herbivorous animals can endure very much larger doses of benzol and benzoates than carnivorous animals.

All rabbits before being subjected to experimentation were observed for a number of days. During this time their blood count was taken daily to establish the individual normal blood picture since it is well known that the "normal" varies with individual rabbits (Klieneberger and Carl, also Moss and Brown). All animals were fed on the same general diet at regular hours. Blood was taken at definite hours to make sure that metabolic influences would be reduced as much as possible. The weight was checked at short intervals. Rabbits with unstable blood pictures were eliminated before experimentation was begun.

In the differential counts no attempts were made to subclassify the various forms of small mononuclear cells encountered. In the same way all the polymorphonuclear cells were grouped under one head, and the remaining large mononuclear, transitional cells and the very rare myelocytes were charted collectively as "large mononuclears." In all instances two smears were counted for two hundred cells each and the average obtained. The percentage was then figured into actual numbers of cells to make the curves more comprehensive. The markings used for curves are uniform on all charts.

The total number of leucocytes is represented by a *very heavy line*. The polymorphonuclear cells are indicated by a *fine line*, the small mononuclear cells by a *broken line* and the "large mononuclears" (including large mononuclear, transitional cells and myelocytes) by a *dotted line*. The presence of snuffles is indicated by *small crosses*.

We do not offer any charts for either the red cell count nor for the hemoglobin, since we found that their stability is not disturbed beyond the normal variations. Neither has the weight been graphically recorded. It usually falls slightly during the first part of the experiment and slowly returns to normal.

We call attention to the constant variations in the appearance of the various types of white cells. Most marked is this in the small mononuclear group. It, generally, holds true that they increase in size but lose their staining affinity considerably when they increase in numbers. Their size at times approaches that of the true large mononuclear cells. With a decrease in the general number they become very small and then develop a marked affinity for the basic stains. Myelocytes appear very rarely and only when the number of leucocytes begins to drop below the normal. Transitional cells always form the majority of the cells grouped together in our third group. They are most numerous when the leucocyte curve reaches its peaks. The polymorphonuclear cells of the rabbit's blood are amphophile in their staining affinity. Therefore, no attempt was made to distinguish between the various subgroups. Basophile cells are easily recognized, but since their number is quite stationary and since they increase only slightly when the total number of leucocytes rises they were counted in with the general "Polymorph group."

EXPERIMENTS AND RESULTS

Group I

Experiment I (see chart I). 0.05 gram of benzyl benzoate were given subcutaneously in sterile olive oil. The dosage used is figured on the basis of that used in men. Local reaction did not occur.

A decided leucocytosis appears in a few days after the daily administration of the drug. The peak or climax is reached rapidly but is not maintained, and there is an even but slow drop which in about one week ends in a mild leucopenia. The differential count is disturbed very little. The dosage used in this experiment is very small for a herbivorous animal and in reality does not correspond to the dose used in men as far as action is concerned.

Experiment II (see chart I). This experiment was carried out in the same way as the one above but the dosage was doubled.

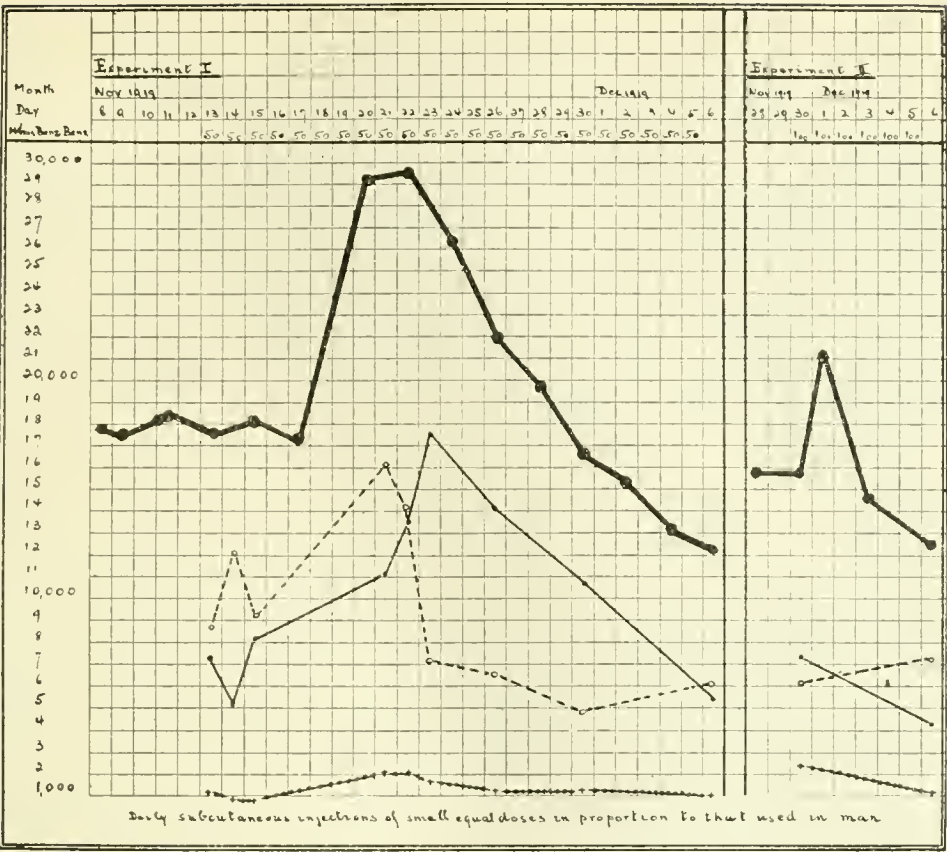


CHART I

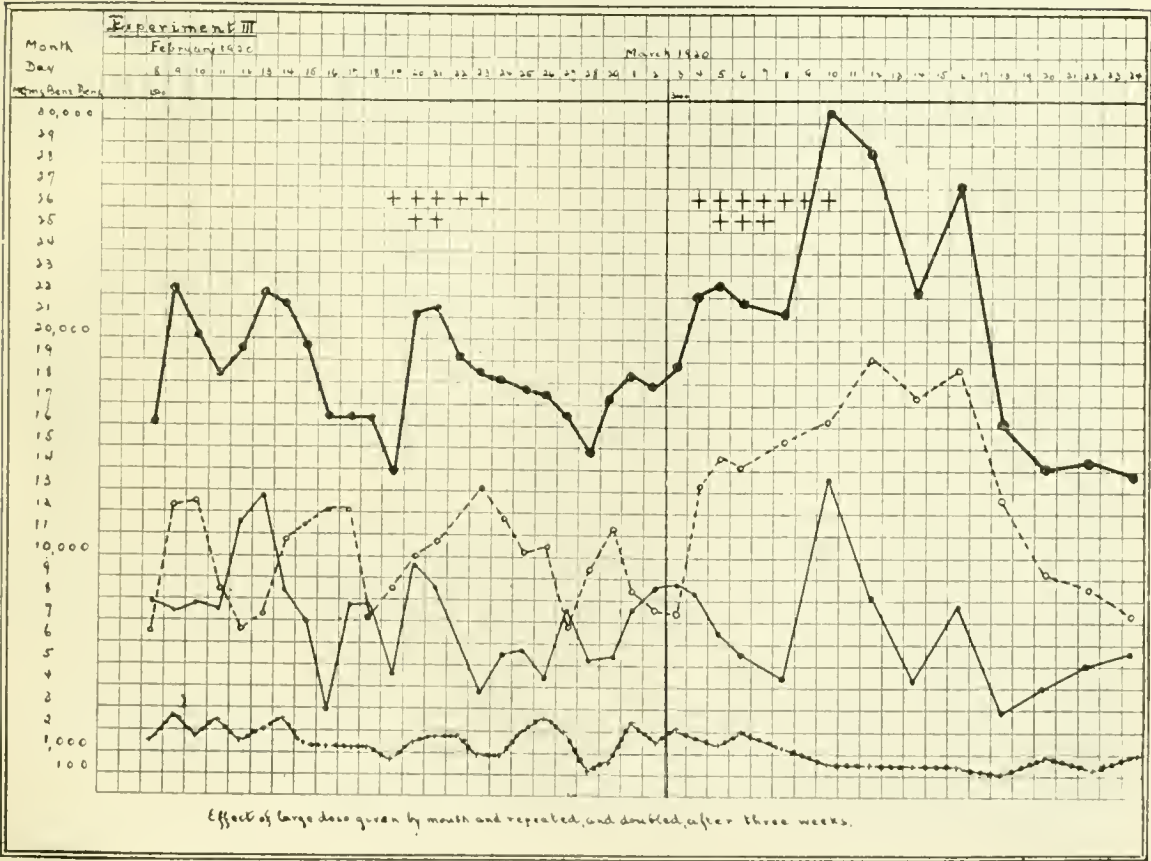


CHART II

The general picture remains the same but the leucocytosis appears more rapidly, the peak is reached with greater suddenness and the break into the downward curve appears more quickly. In other words, a leucopenia is produced more suddenly. In the differential count the small mononuclear cells rise at once over the polymorphonuclear element.

Group II

Experiment III (see chart II). In order to determine the effect of a single large dose of benzyl benzoate, 1.5 grams were given per stomach tube. The rise in the total count occurs at once. With it, there is a sharp increase in the lymphocytic element which ultimately develops into a lymphocytosis. There is a distinct diphasic reaction in the total count of white cells but its curve is exactly the reverse of that of benzol. Also here, at the end of one week a mild leucopenia at the expense of the polymorphous element occurs. It was at this point that the animal began to suffer from "snuffles" which was followed by a renewed rise in the total number of leucocytes. At first, this was a true polymorphonuclear leucocytosis but developed into a lymphocytosis as the count once more approached the "sub-normal."

With the return of the white blood cells to the normal baseline the animal seemed to have overcome its infection, at least it was symptomatically free from "snuffles." Then, a second and double dose of benzyl benzoate was administered to see if the infection would light up again. On the following day the animal suffered again and more severely from "snuffles." There appeared also a marked rise in the total leucocyte count with a steady increase in the small mononuclear element. The total picture is the same as in the first part of this experiment but its characteristics are very much more pronounced. The diphasic phenomenon again was present in the second part of the experiment. Also here a late leucopenia occurred but appeared more suddenly than in the first part of the experiment.

Experiment IV (see chart III). This experiment was carried out with the idea that if free benzol was present in the breaking down of large doses of benzyl benzoate, a white cell curve similar to that of benzol should occur.

Five grams in olive oil were given by stomach tube. There were no symptoms whatsoever to suggest that a toxicosis was produced. There was neither vomiting nor diarrhoea. The rabbit was lazy and the vessels of the ears were greatly distended. This phenomenon was observed practically after each large dose in other experiments.

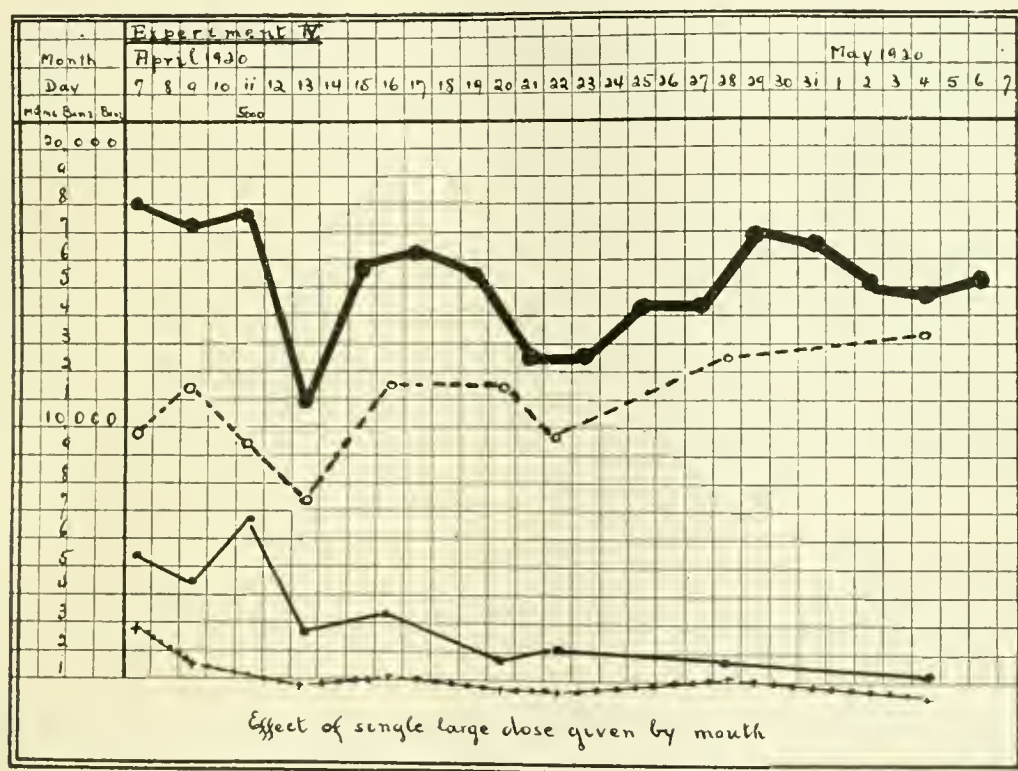


CHART III

There appeared at once a true leucopenia which produced a diphasic curve somewhat the same in general appearance to that of Weiskotten's benzol curve. The leucopenia is also here at the expense of the polymorphonuclear element and is very severe. With the rise of the small mononuclear cells, myelocytes appear in excess over both the large mononuclear lymphocytes and transitional cells. The small mononuclear cells stain with difficulty

and become very irregular in shape and size. At the end of this experiment, that is after almost one month had elapsed, the normal blood picture had not yet reappeared in spite of the fact that the total count had returned to the normal base-line for about ten days.

Group III

This group of experiments was carried out to determine if either the method of administration of the drug or the vehicles used would influence the general blood picture.

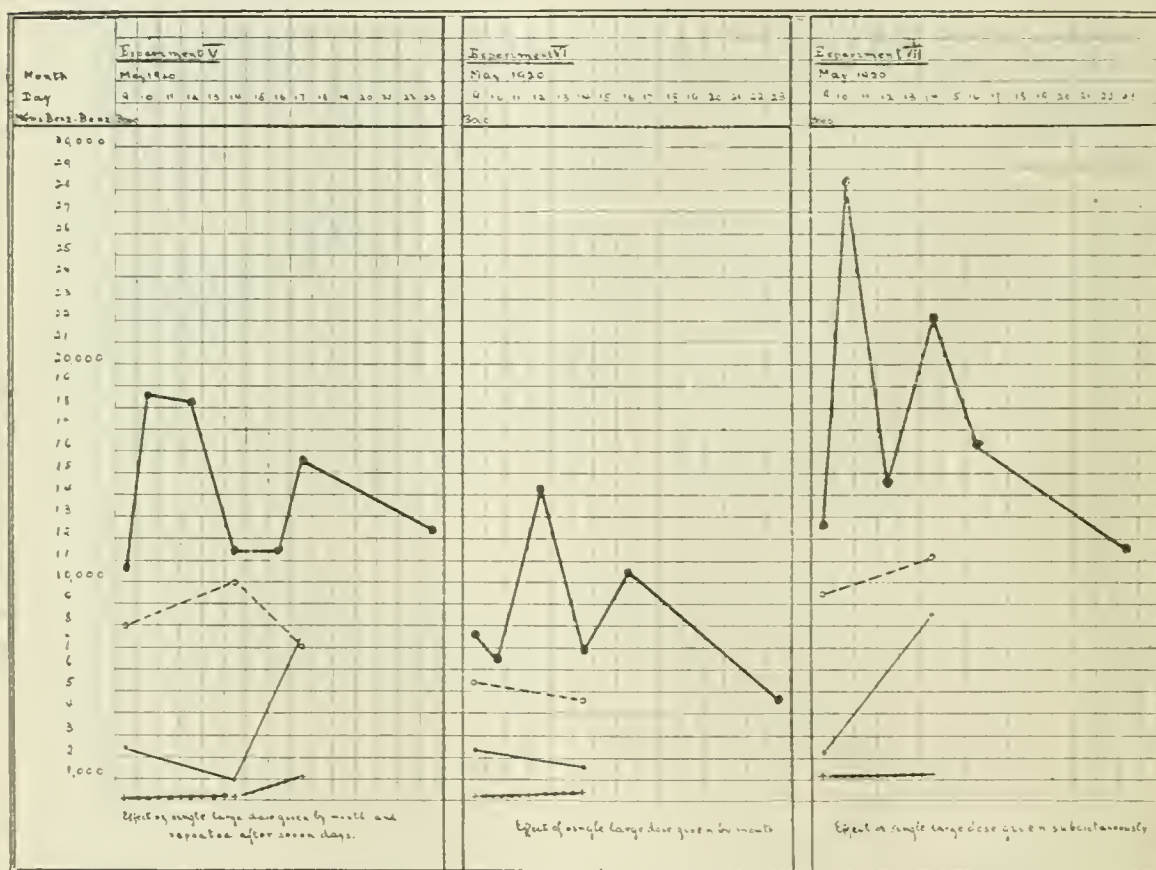


CHART IV

Experiments V, VI and VII (see chart IV). The same dose, 3 grams was given in all three experiments. In experiment V it was given in dilute alcohol by stomach tube, in experiment VI in olive oil by the same method and in experiment VII it was injected subcutaneously.

Except that the curves lie at different levels due to the individual variation of the "normal," they are otherwise in every

respect similar in their general behavior. There is always the diphasic leucocytosis which ultimately ends in a late leucopenia. In the subcutaneous injection the reaction is perhaps more severe.

Aside from general lassitude and loss of appetite none of the animals had any ill symptomatic effect of the comparatively large dose. The weight decreased at an average of about 20 per cent but was regained rapidly toward the end of the observation.

The differential count was not followed closely enough to determine if the behavior of the various elements was affected in a similar manner as noted previously.

Experiments VIII, IX, X (no chart). In this group the effect of the vehicles was determined by injecting olive oil daily subcutaneously, administering it by mouth in the same amount to another animal and comparing the two to a third animal which had been given dilute alcohol of various strengths. Olive oil given either way does not change the blood picture whatsoever and alcohol produces a very transient depression of the normal count but without changing the differential arrangement of the cells. We conclude therefore that the vehicles used in our experiments do not exert any influence on the blood picture of the rabbit, and consequently, any changes that do occur must be laid to the drug used.

Group IV

In this group it was attempted to demonstrate that benzyl benzoate depresses the anti-infectious powers of the body perhaps in a similar way to benzol which permits quiescent or latent infections to become acute.

Experiment XI (see chart V). This rabbit which had previously suffered from "snuffles" was symptomatically free from the disease at the time of this experiment. The blood count though, was above the normal upper limit and therefore it was counted daily until it had reached a stationary low level which we considered the base line. The rabbit was then given 1 gram of benzyl benzoate by mouth. There followed an immediate outbreak of "snuffles." The leucocytes rose enormously with the polymorphous element distinctly predominating. After the

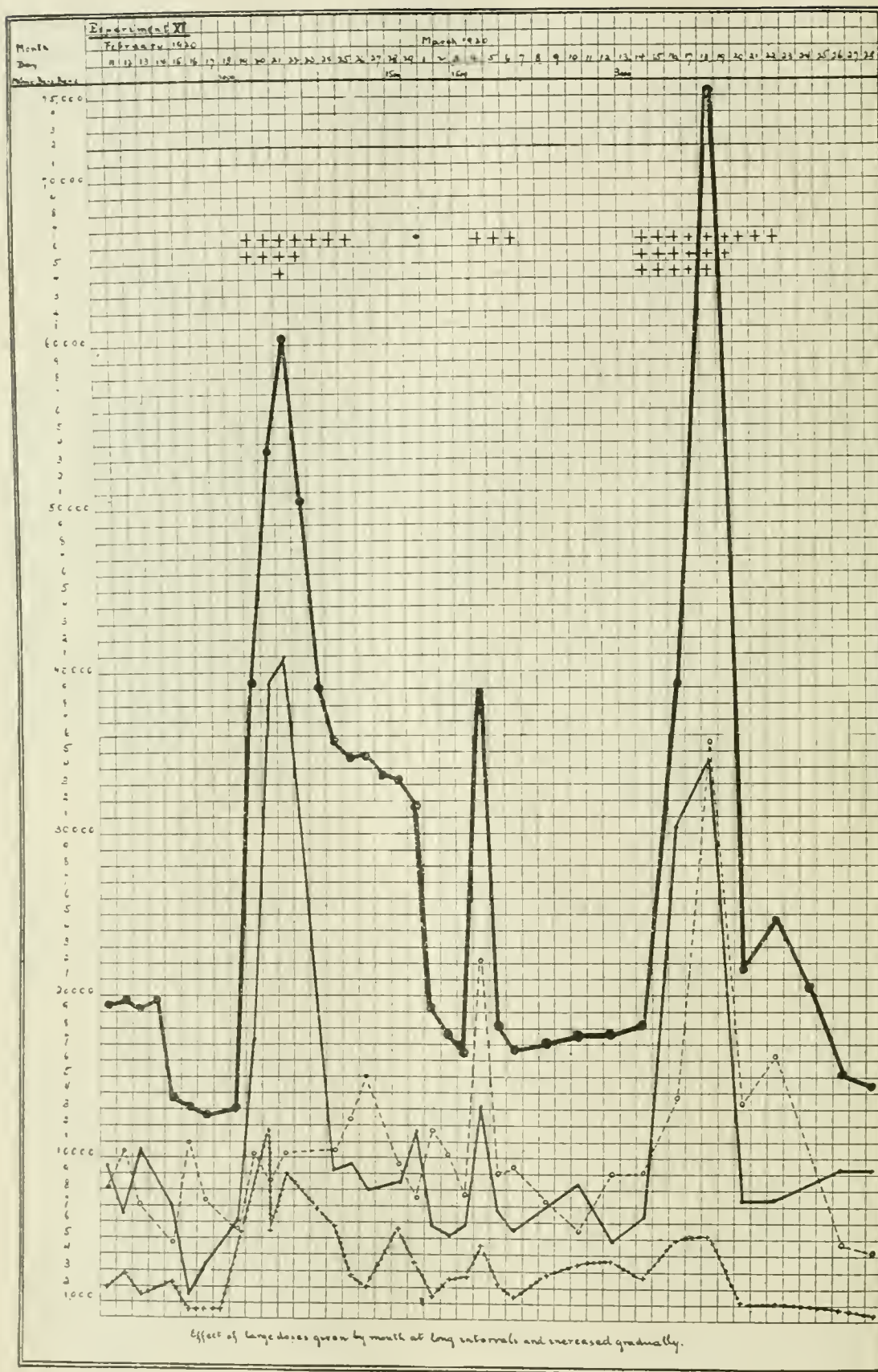


CHART V

symptoms had subsided and the leucocyte curve was on the downward run, another dose, this time 1.5 gram, was given by mouth with the result that the count dropped to almost normal

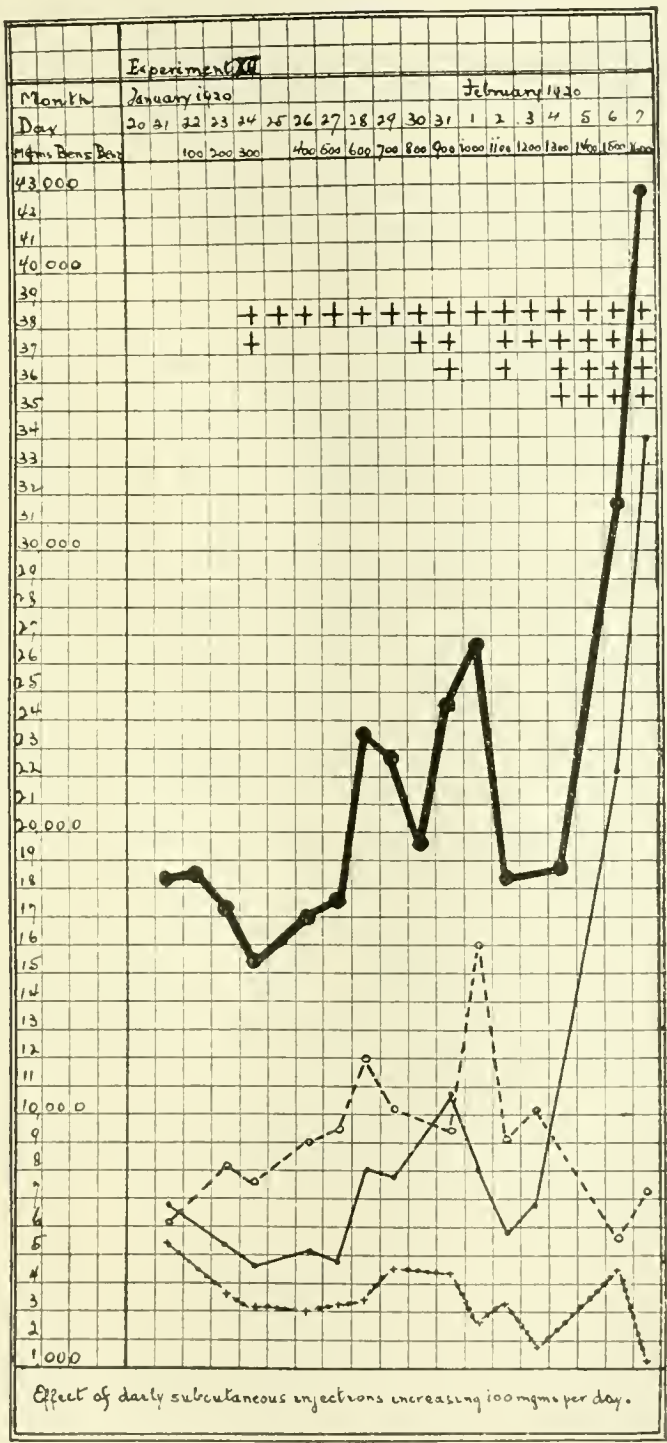


CHART VI

level. There was now a predominance of small mononuclear cells in the differential picture. Two more doses, one of 1.5 grams and the other of 3 grams were given and the leucocyte curve rose each time but very much more spectacularly with the last dose. Each time also the disease broke out fresh and the animal almost succumbed three days after the last dose and after the leucocyte curve was near the normal base line. It is interesting that the small mononuclear cells remained in the majority and rose well above the polymorphs with each of the last three administrations.

Experiment XII (see chart VI). Another rabbit which also suffered mildly from "snuffles" but which otherwise was fat, lively and of smooth hair, was given a daily subcutaneous injection of benzyl benzoate, beginning with 100 mgm., and adding an equal amount each day. A diphasic leucocytosis occurred with the small mononuclear element predominating. "Snuffles" recurred after three days when the animal was given a rest. With each subsequent dose the animal snuffled more severely. After the tenth injection when the dose had reached a 1000 mgm. the affliction became very acute, the leucocytosis broke and remained so for two days, in spite of increased dosage, and then suddenly entered into an extreme leucocytosis with the polymorphs far outnumbering the small mononuclears. The animal died, we think rather from "snuffles" than from the benzyl benzoate intoxication but we also believe that the latter substance was responsible in depressing the vital forces of the body of the rabbit to such a degree that the disease became overwhelming, because rabbits are otherwise very resistant to "snuffles," at least as far as mortality is concerned. We include under "snuffles" any of the nasal infections of rabbits.

CONCLUSIONS

There are certain outstanding facts which have recurred in most of our experiments and the observation of which would most likely have been missed if our experiments would have been limited to shorter periods. The limited number of our experi-

ments is offset by uniform results. These lead us to believe that in rabbits a continuous administration of benzyl benzoate in small doses leads to a leucocytosis which in somewhat larger doses is accompanied by an increase in small mononuclear cells. This leucocytosis is transient and ultimately ends in a late mild leucopenia. In other words the behavior of the blood picture suggests also that if it is due to some form of benzol or some intermediate product, such substance is of importance only after a sufficient amount of benzyl benzoate has accumulated in the system.

In large but single doses of benzyl benzoate there is a tendency to change the even rise of the leucocytes into a broken curve of a diphasic character with a distinct depression of the polymorphonuclear element. Also here the mild and late leucopenia occurs. The changes in the blood curve are not dependent upon the method of the administration of the drug. In a very large, single, but not fatal dose the primary rise of the leucocytes does not occur necessarily but the blood curve assumes more of the leucopenic character, of the benzol curve. This leucopenia presents also here a diphasic polymorphonuclear picture.

In the presence of latent or quiescent infections in rabbits larger doses produce an acute return of the disease. This is accompanied by sharp rises and sudden drops in the total as well as the small mononuclear white blood cells (actual lymphocytosis). A leucopenia was not observed when the recrudescence was very violent but when the recrudescence took a milder course there also was a suggestion of a late leucopenia. The flaring up of an infection in rabbits during benzyl benzoate therapy suggests a similarity to the action of benzol under similar circumstances.

That our data are in direct contradiction of those of Heller and Steinfield, whose report appeared after the completion of this investigation does not necessarily invalidate one or the other. It was stated by them that benzyl benzoate has "no toxic effect upon the leucocytes of the rabbit." We feel, though, that Heller and Steinfield's statements are too sweeping in character

since with one exception they are based on single injections of benzyl benzoate. They have not taken into consideration that there may be an accumulative action of the drug, nor have they carried on their experiments over a sufficiently long period. The absence of differential counts in their report makes their observations on the blood picture incomplete. Their statement that there is a wide margin between the "therapeutic dose of benzyl benzoate used in men and that given to rabbits," is misleading. Men, being omnivorous animals, may respond differently to benzyl benzoate than herbivorous animals. The latter are capable of breaking down benzoates with greater ease than carnivora, and consequently, we may expect rabbits to take care of considerably larger doses than men. In order to establish a true ratio, comparative experiments on both groups of animals must be undertaken.

The small number of our clinical observations does not permit us to make definite statements. We have no early blood counts in two patients and therefore do not know if a primary leucocytosis occurred. On the strength of our experimental results we assume for the present that the low number of white cells may have been due to the action of benzyl benzoate and that possibly the recrudescence of the appendicite inflammation was due to a similar influence.

In regard to the red blood cells and the hemoglobin we found that benzyl benzoate has no or only a very slight depressing influence. We do not offer any explanation for the peculiar changes observed in the size and staining intensity of the small mononuclear cells but merely state the fact that they increase in size with any sudden rise of the total number of this group and became very small when the mononuclear curve is on the descent. The initial loss of weight noted in most of our experiments is not usual in any rabbit experimentation and since there is a compensatory return to the normal, we do not lay any stress upon it.

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ROUTE OF ADMINISTRATION OF DRUGS IN RELATION TO TOXICITY IN CHEMOTHERAPEUTIC INVESTIGATIONS WITH SPECIAL REFERENCE TO INTRAPLEURAL INJECTIONS OF ETHYLHYDROCUPREIN HYDROCHLORID¹

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In experiments upon the treatment of experimental pneumococcus meningitis in rabbits with ethylhydrocuprein (optochin) hydrochlorid and mercuraphen injected subthecally, Dr. Idzumi and myself (1) found these drugs from eight to ten times more toxic than when injected intravenously. More recently Mr. Sands and myself (2) have used ethylhydrocuprein in the treatment of pneumococcus pleuritis produced experimentally in guinea-pigs and dogs and found to our surprise that the drug was slightly more toxic than by intravenous injection.

These results suggested the advisability of studying more closely the toxicity of several drugs by different routes of administration and especially by intrapleural injection.

It may be stated here that ethylhydrocuprein hydrochlorid had a distinct curative influence upon the course of pneumococcus meningitis in rabbits when injected into the subarachnoid space of the spinal meninges within four to six hours after infection; when treatment was delayed for twenty-four to forty-eight hours, there were slight or no beneficial results. In experimental pneumococcus pleuritis in guinea pigs even better effects were observed. Control animals usually succumbed within seventy-two hours after the intrapleural injection of pneumo-

¹ Investigation aided by funds accruing from the preparation of arsphenamine.

² I am indebted to Miss Anna Rule for assistance in this investigation.

coccus culture with severe bilateral purulent pleuritis and pericarditis and bacteremia. The injection of ethylhydrocuprein hydrochlorid into *both* pleural sacs within twenty-four hours after infection and in doses from two and a half to five times less than the toxic amounts, saved the majority of the animals for indefinite periods of time. *These experiments have opened up the possibility of drugs possessing high bactericidal activity for a particular bacterium in the presence of pus, being used in the treatment of infections of closed sacs even though the toxicity of the medicament is such that it cannot be injected intravenously in sufficient amounts to effectually destroy bacteria in a focus of infection.*

PURPOSE OF INVESTIGATION

As previously stated my chief purpose was to compare the toxicity of ethylhydrocuprein hydrochlorid injected intrapleurally with toxicity by intravenous, intraperitoneal, subthecal and subcutaneous injection to determine the *highest tolerated doses* according to these different routes of administration.

In order to confirm the results observed other medicaments were studied in the same manner including quinin and urea hydrochlorid, quinin bisulphate, mercurophen, arsphenamin and neoarsphenamin.

Since it would appear that the toxicity of a drug may vary when injected subcutaneously or intravenously according to the animal used, three of the above mentioned compounds namely, ethylhydrocuprein, arsphenamin and neoarsphenamin, have been studied in mice, rats, rabbits and guinea-pigs.

EXPERIMENTAL

White rats weighing from 100 to 200 grams were employed in the majority of experiments; mice, guinea-pigs and rabbits were also employed with some of the drugs.

For intravenous injection the drugs were used in from 0.5 to 2 per cent solutions except in the case of neoarsphenamin when 4 per cent solutions were employed. The rate of injection was always 0.5 cc. per minute by means of a gravity apparatus

previously described (3). Experience has shown quite conclusively that the toxicity of ethylhydrocuprein and other quinin compounds, mercurophen, arsphenamin and neoarsphenamin by intravenous injection is greatly influenced by the rate of injection. This is particularly true of the immediate lethal effects; less so in tests for the highest tolerated doses in seven days. Rapid intravenous injection materially increases the toxic effects of the above mentioned compounds as compared with slow injections of solutions of the same strength.

For intrapleural and intraperitoneal injections the same concentrations were used; the solutions were warmed to 38°C. and injected with a syringe at about 1 cc. per minute.

For subcutaneous injections the solutions were more concentrated and injected more rapidly; usually a dose would be injected under the skin of the back and divided among two or three sites in order to avoid excessive tissue injury resulting from a large amount of fluid injected in one place.

All solutions were prepared in sterile distilled water. The solutions of arsphenamin were alkalized by the addition of 0.9 cc. of normal sodium hydroxid per 0.1 gram of drug.

All of the experiments were repeated at least twice, the majority three times and a few four times in order to obtain the upper and lower limits of toxicity and the average toxicity inasmuch as single experiments seldom yielded acceptable results because of variation in the resistance of animals of the same species injected with the same drug in exactly the same manner.

As previously stated only the highest tolerated doses were determined, that is, the largest amounts per kilogram of weight tolerated for seven days.

RESULTS

Table 1 and charts 1, 2, 3, 4 and 5 give the average highest tolerated doses for white rats of ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, quinin bisulphate, mercurophen,³

³ Mercurophen is sodium oxymercury orthonitro phenolate prepared by Schamberg, Kolmer and Raiziss (4).

arsphenamin⁴ and neoarsphenamin⁴ administered by different routes. Ethylhydrocuprein and mercurophen were also injected subthecally in rabbits.

Without exception all of the drugs included in this study were slightly more toxic by intrapleural than by intravenous injection.

Judging according to the results observed with ethylhydrocuprein and mercurophen, drugs produce most injurious effects

TABLE 1
Summary showing highest tolerated doses in seven days of various drugs administered by different routes to white rats

COMPOUND	ANALYSIS	PER KILOGRAM OF WEIGHT				
		Subcutaneous	Intra-peritoneal	Intra-pleural	Intra-venous	Sub-thecal (rabbit)
Ethylhydrocuprein hydrochloride	75-80% alkaloid	0.500-0.550	0.150	0.020-0.040	0.030-0.040	0.003-0.005
Quinine and urea hydrochloride	58% alkaloid	More than 0.800	0.200	0.040	0.065	
Quinine bisulphate	59.1% alkaloid	More than 0.800	0.200	0.040	0.065	
Mercurophen	52% Hg	About 0.080	0.020-0.030	0.003-0.008	0.008	0.001
Arsphenamine	31% As	0.400-0.600	0.200-0.350	0.080-0.120	0.110-0.140	
Neoarsphenamine	19% As	0.150	0.240-0.300	0.070-0.090	0.240-0.280	

upon subthecal injection, next by intrapleural, intravenous, intraperitoneal and subcutaneous injection named in order. An exception to this general rule is noted with neoarsphenamin which curiously, is not as well borne by subcutaneous injection

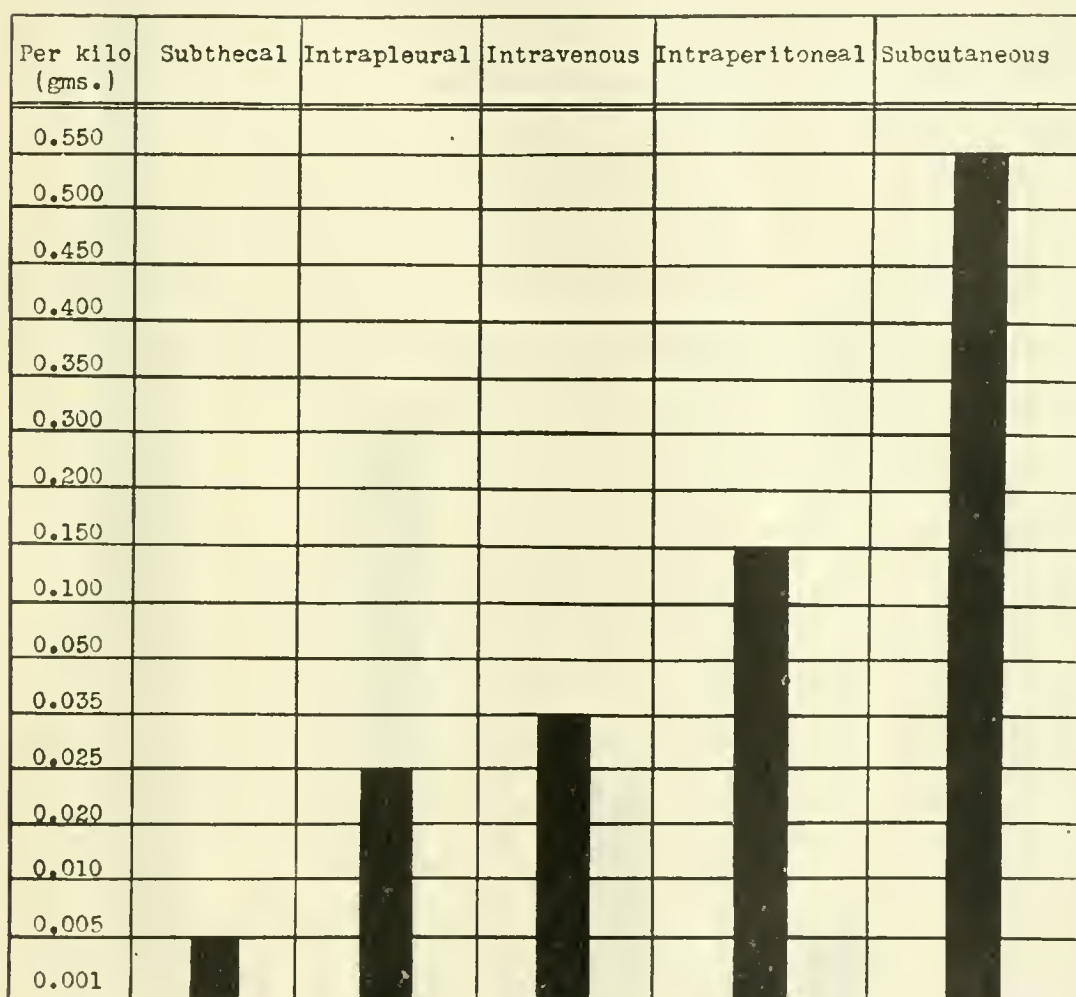
⁴ Figures were based upon tests with three different arsphenamins and neoarsphenamins prepared by the Dermatological Research Laboratories of Philadelphia.

as by intravenous and intraperitoneal injection (chart 5). This was first described by Castelli (5) and confirmed by Schamberg, Kolmer and Raiziss (3) and the present investigation.

It is believed that the figures given are approximately correct; highest purity products were used but even these vary in toxicity

CHART 1.

Highest Tolerated Doses of Ethylhydrocuprein
Hydrochlorid Per Kilogram of White Rat



as for example, different lots of arsphenamin and neoarsphenamin as shown by Roth (6), Schamberg, Kolmer and Raiziss (3) and others.

Table 2 shows how the highest tolerated doses by subtheccal, intrapleural, intraperitoneal and subcutaneous injection com-

CHART 2.

Highest Tolerated Doses of Quinin and Urea Hydrochlorid
and Quinin Bisulphat Per Kilogram of White Rat.

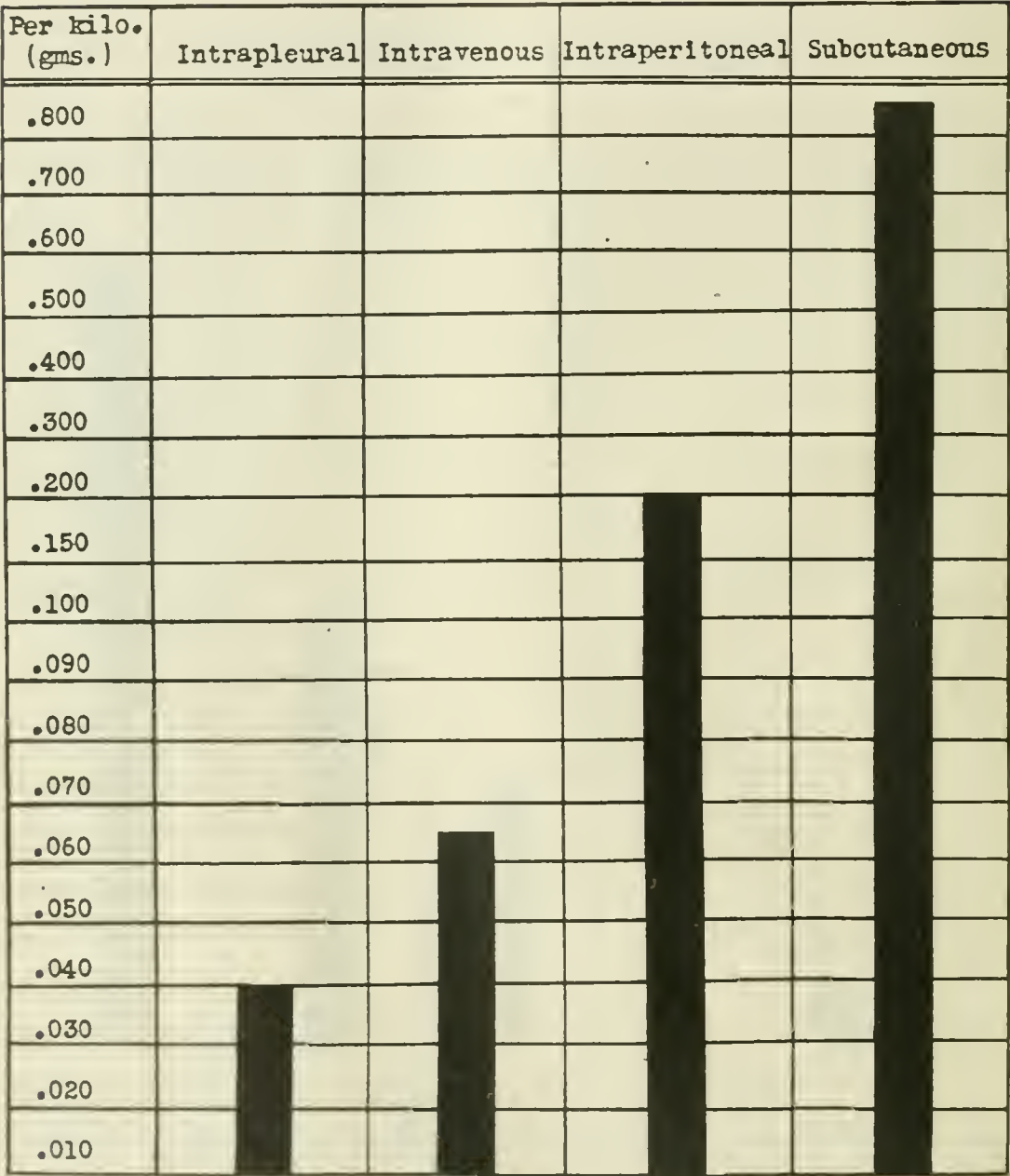
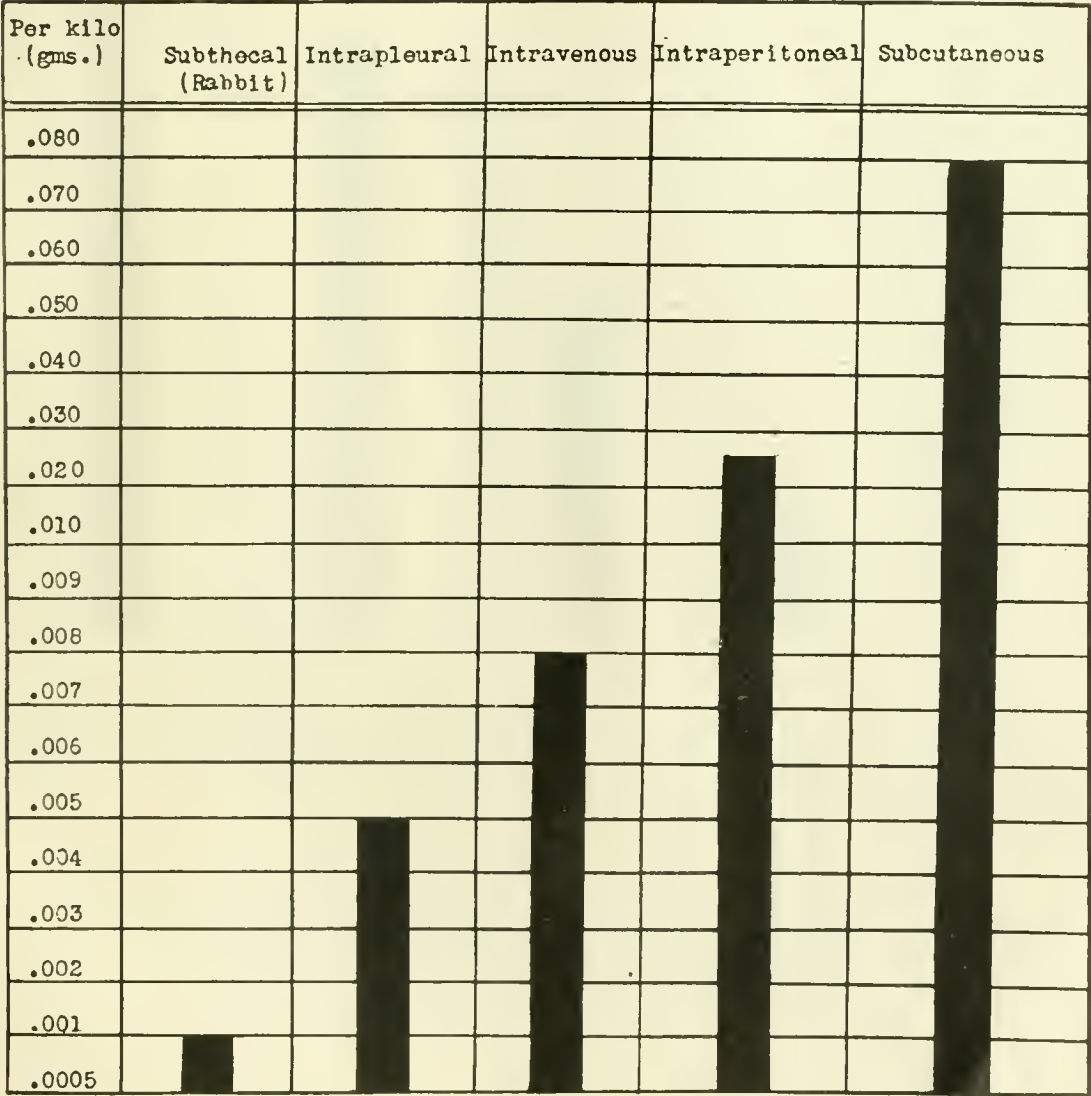


TABLE 2
Highest tolerated doses by subthechal, intrapleural, intraperitoneal and subcutaneous injection compared with intravenous injection

COMPOUND	SUBTHECAL	INTRAPLEURAL	INTRAPERI-TONEAL	SUBCUTANEOUS
Ethylhydrocuprein	7 × less*	0.70 × less	4.3 × more†	16 0 × more
Quinine and urea hydrochloride . . .		0.61 × less	3.1 × more	13.0 × more
Quinine bisulphate		0.60 × less	3.0 × more	13 0 × more
Mercuriophen		0.63 × less	3.0 × more	10.0 × more
Arsphenamine		0.66 × less	1.7 × more	2 0 × more
Neorsphenamine		0.32 × less	1.1 × more	0 6 × less

* *Less* means highest tolerated dose was so many times less or smaller than highest tolerated dose by intravenous injection.
† *More* means highest tolerated dose was so many times more or greater than highest tolerated dose by intravenous injection.

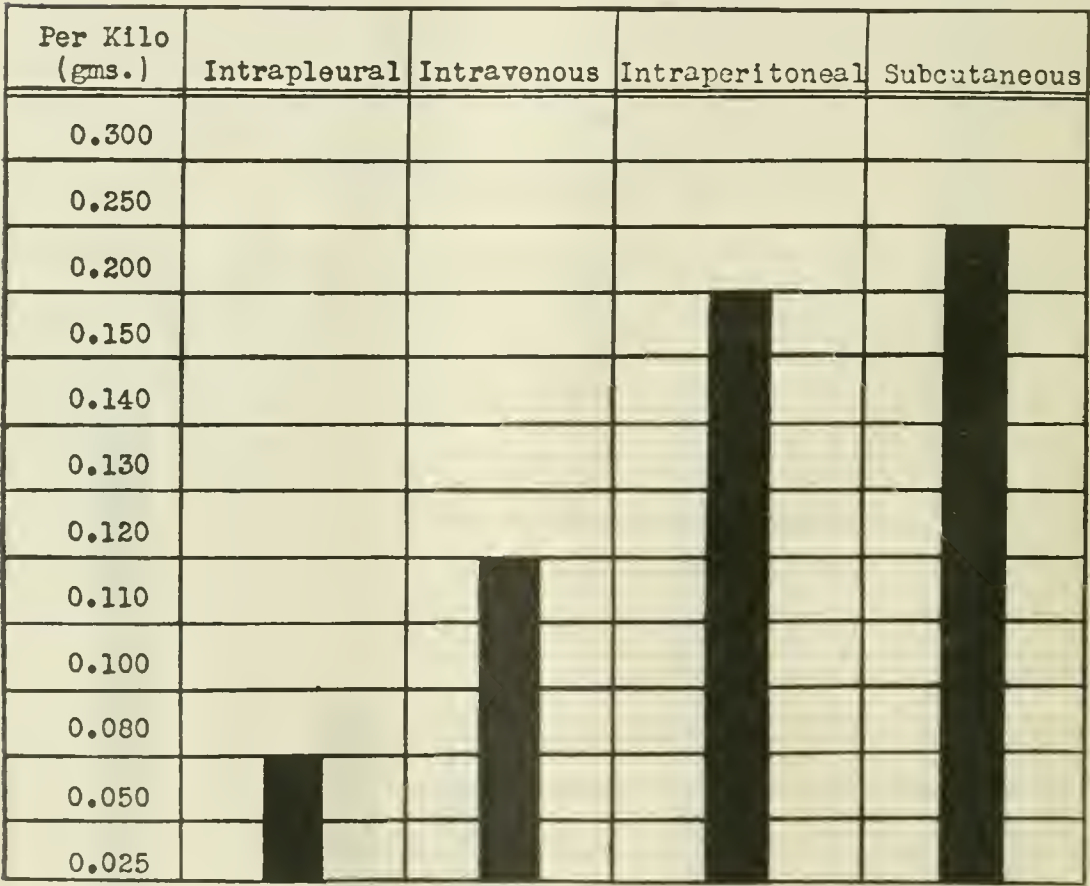
CHART 3.
Highest Tolerated Doses of Mercuriophen
Per Kilogram of White Rat



pared with those by intravenous injection. For example, the highest tolerated dose of ethylhydrocuprein by subtheal injection was 7 times less and 16 times more by subcutaneous injection, as compared with the results of intravenous injection.

CHART 4.

Highest Tolerated Doses of Solutions of Disodium
Arsphenamin Per Kilogram of White Rat



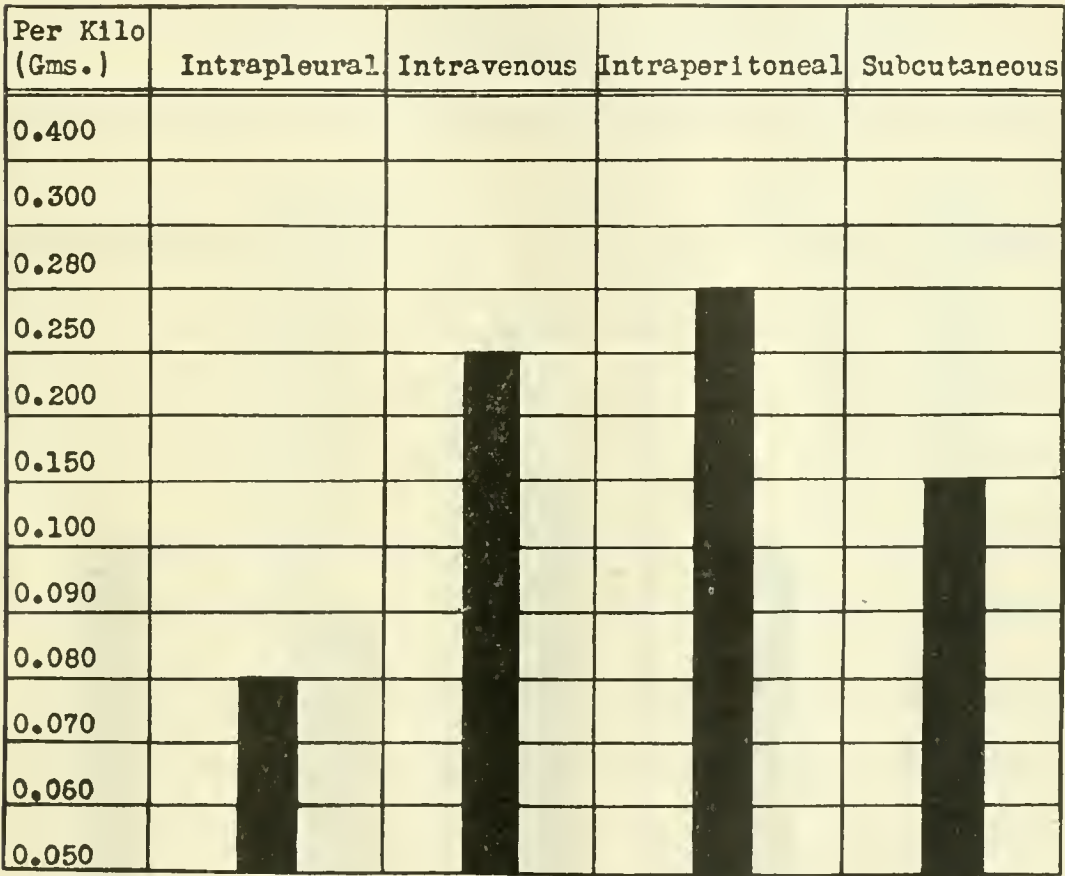
In general terms the toxicity of quinin compounds (including ethylhydrocuprein) and mercurophen compared with toxicity by intravenous injection, was as follows:

- 7 to 8 times more toxic by subtheal injection
- 0.6 to 0.7 times more toxic by intrapleural injection
- 3 to 4 times less toxic by intraperitoneal injection
- 10 to 16 times less toxic by subcutaneous injection

In a study of the comparative toxicity of various preparations of mercury Schamberg, Kolmer and Raiziss (7) found the average relationship between intravenous and intramuscular administration to be about 4 to 1.

CHART 5.

Highest Tolerated Doses of Neoarsphenamin
Per Kilogram of White Rat



With arsphenamin the values were as follows:

- 0.6 to 0.7 times more toxic by intrapleural injection
- 1 to 2 times less toxic by intraperitoneal injection
- 2 times less toxic by subcutaneous injection

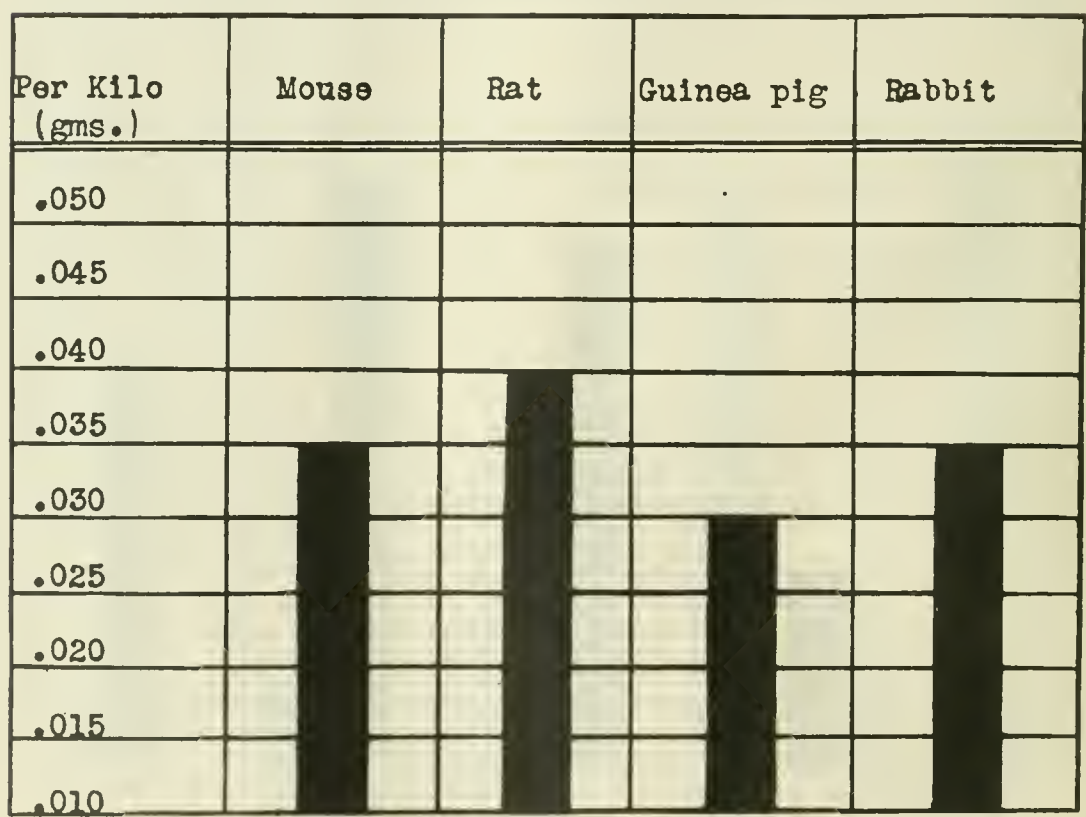
With neoarsphenamin the values were as follows:

- 0.3 to 0.4 times more toxic by intrapleural injection
- 1 to 1.5 times less toxic by intraperitoneal injection
- 0.5 to 1 times more toxic by subcutaneous injection

For the mouse Castelli (5) found arsphenamin 1.7 times less toxic by subcutaneous than by intravenous injection and neoarsphenamin about 3 times more toxic by subcutaneous than by intravenous injection. Schamberg, Kolmer and Raiziss (3) found arsphenamin about 3 times less toxic for the white rat by subcutaneous injection than by intravenous injection and

CHART 6.

Highest Tolerated Doses of Ethylhydrocuprein Hydrochlorid by Intravenous Injection



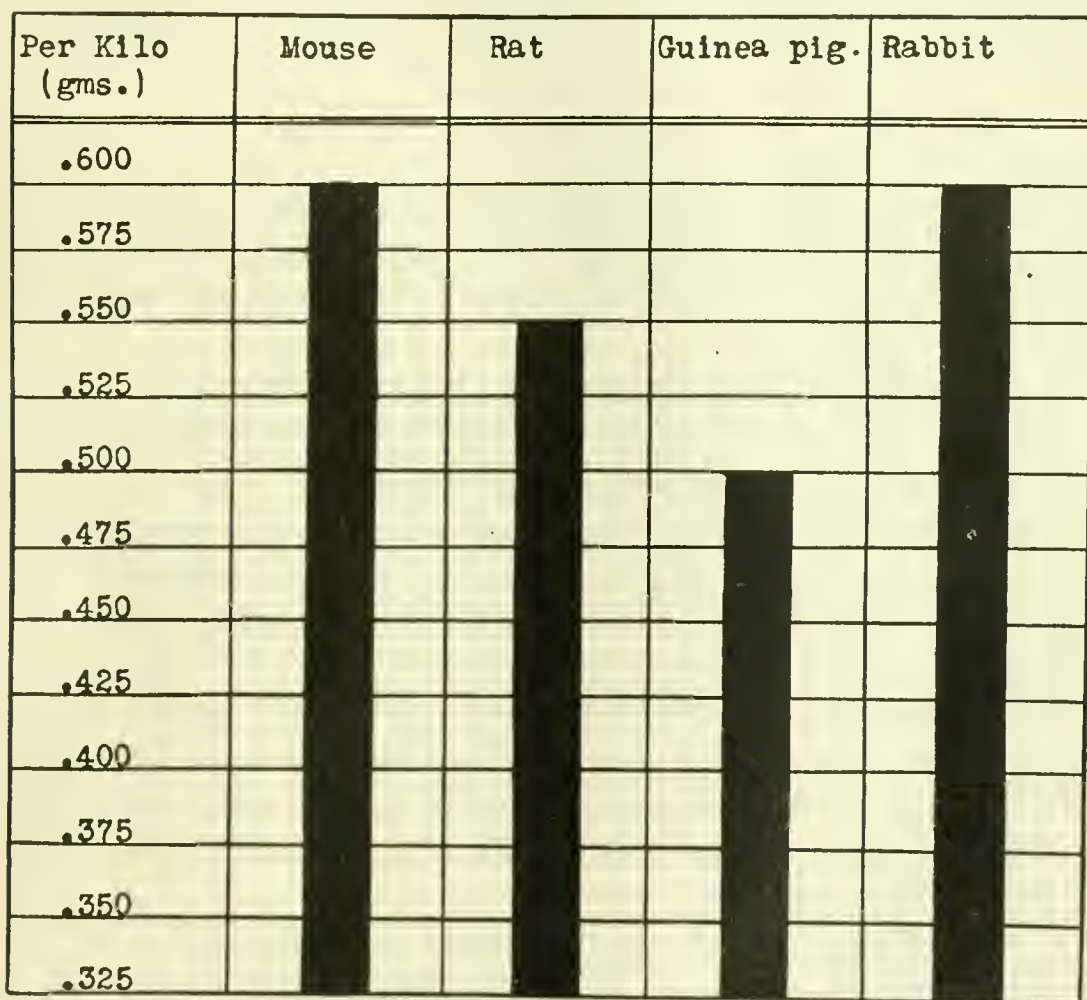
neoarsphenamin about 0.6 to 0.7 times more toxic by subcutaneous than by intravenous injection.

It is quite evident therefore, that there is no constant or uniform relationship between the highest tolerated doses of different drugs according to the route of administration. It may be stated however, that in general terms a substance is apt to be 7 to 8 times more toxic when injected subthecally than intra-

venously; 0.6 to 0.7 times more toxic by intrapleural injection and 2 to 16 times less toxic by subcutaneous injection. One substance namely, neoarsphenamin, proved more toxic by subcutaneous than by intravenous injection.

CHART 7.

Highest Tolerated Doses of Ethylhydrocuprein
Hydrochlorid by Subcutaneous Injection



Apparently the toxicity of arsphenamin and neoarsphenamin varies according to the test animal, the smaller the animal the greater the tolerance. For example the mouse can stand larger amounts of these compounds by intravenous injection according to body weight than the white rat and the rat more than the rabbit.

With ethylhydrocuprein hydrochlorid these differences are not nearly so marked.

Chart 6 shows the highest tolerated doses per kilogram of weight of ethylhydrocuprein administered by intravenous injection to mice, rats, guinea-pigs and rabbits; the doses were remarkably uniform being from 0.030 to 0.040 gram per kilo.

Chart 7 shows the variation in highest tolerated doses of ethylhydrocuprein for these animals by subcutaneous injection, the values ranging from 0.500 gram per kilogram of pig to 0.550 gram for the rat and about 0.600 gram for the mouse and rabbit.

SUMMARY

1. Ethylhydrocuprein hydrochlorid, other quinin compounds, mercurophen, arsphenamin and neoarsphenamin were 0.6 to 0.7 times more toxic for white rats by intrapleural than by intravenous injection.

2. Ethylhydrocuprein hydrochlorid and mercurophen were about 7 to 8 times more toxic for rabbits by subthecal injection than for white rats by intravenous injection.

3. Ethylhydrocuprein hydrochlorid, other quinin compounds and mercurophen were 3 to 4 times less toxic for white rats by intraperitoneal injection than by intravenous injection; arsphenamin and neoarsphenamin were 1 to 2 times less toxic.

4. Ethylhydrocuprein hydrochlorid, and other quinin compounds and mercurophen were 10 to 16 times less toxic for white rats by subcutaneous injection than by intravenous injection; arsphenamin was 2 times less toxic but neoarsphenamin was 0.5 to 1 times more toxic.

5. The toxicity of ethylhydrocuprein hydrochlorid by intravenous injection to mice, rats, guinea-pigs and rabbits was quite uniform the highest tolerated doses being 0.03 to 0.04 gram per kilogram of weight. Toxicity by subcutaneous injection varied from 0.500 gram per kilogram in the guinea-pig to 0.600 gram per kilogram in the mouse and rabbit.

6. The toxicity of arsphenamin and neoarsphenamin by intravenous and subcutaneous injection varies with the test

animal, the highest tolerated doses being observed with mice, next with rats and rabbits in the order named.

7. There is no constant or uniform relation among the highest tolerated doses of different compounds for animals of the same species with the same route of administration. In very general terms a compound is apt to be about 8 times more toxic by subthecal than by intravenous injection; 0.6 to 0.7 times more toxic by intrapleural, 1 to 4 times less toxic by intraperitoneal and 2 to 16 times less toxic by subcutaneous, than by intravenous injection.

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COMPARATIVE EFFECTS OF MORPHIN AND ALKALOIDS OF THE BENZYLISOQUINOLIN GROUP ON CARDIAC MUSCLE

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I. INTRODUCTION

The current classification of the opiate alkaloids into the pyridin-phenanthrene and benzylisoquinolin groups suffers a lack of detail in order to be fundamentally applicable and comprehensive. The pharmacological distinction between these two groups of alkaloids rests chiefly upon their opposite effects on smooth muscle, namely, a stimulant action by the pyridin-phenanthrene group and a depressant action by the benzylisoquinolin group. Concerning their actions on other functions and organs, the evidence is incomplete and confusing. This includes cardiac muscle, which is unusually suitable for investigations of this sort, not to mention the practical importance of the results in the circulatory effects of these agents.

This has been made the special object of this report, using the perfused amphibian heart as test object. The alkaloids studied were morphin (pyridin-phenanthrene) and chelidonin, papaverin

and narcotin (benzylisoquinolin) and the related alkaloids, hydrastin, hydrastinin and cotarnin. Of these morphin and papaverin have been studied previously by several investigators, but the remaining agents either have been studied imperfectly or not at all. Before proceeding to the main body of the report it will be of interest to present a brief abstract of some of the investigations bearing more or less directly on the subject under consideration.

The results of the older investigators on perfused hearts are summarized in a paper by Worth Hale (1), who studied the effects of the opiate alkaloids on the atropinized frog's heart, using the changes in rate and cardiac output as index of their activity. The results of Hale and of his predecessors are not altogether uniform and also susceptible of different interpretations. In part this lack of uniformity may be due to the variable and inadequate concentrations of the drugs employed. In my work a range of higher concentrations was used in order to secure definite effects. The older studies include no observations on the effects of hydrastinin, hydrastin and narcotin on the perfused heart as used in my work. Of these agents hydrastinin is very closely related to hydrastin, which is used for the preparation of hydrastinin. Cotarnin is derived from narcotin. Therefore, a similarity in pharmacological action of these agents might be expected. As for cardiac muscle, only the effects of narcotin are known. This was found by Hale to be a powerful depressant. Hydrastinin, hydrastin and cotarnin have been especially studied by Macht (2) for their effects on the smooth muscle of the ureter. Macht claims that hydrastinin and cotarnin possess a morphin-like (pyridin-phenanthrene) or stimulant action and hydrastin a papaverin-like (benzylisoquinolin) or depressant action on the ureteral muscle. However, the published records of this author do not appear convincing, although, of course, the analysis of all his results may still justify his classification. In a subsequent paper by Macht (3), it appears that the ureteral effects of hydrastinin and hydrastin are less uniform than those on the uterus, intestine, artery, bladder and vas deferens of different species. As a matter of fact the pharmacological classification of

these agents by the ureteral effects is only partially sustained by the results on the heart presently to be described. Before considering these it will be of interest to mention a recent paper by P. Trendelenburg (4), which points out certain important conflicts between his results and prevalent notions concerning the action of morphine on intestine. This bears directly on the usefulness and accuracy of the modern classification of the alkaloids of the papaveraceae.

By an improved method of studying the activity of excised intestine, Trendelenburg shows that very low concentrations of morphin depress the peristalsis of guinea-pig intestine, in much the same way as papaverin acts, and also as morphin acts on human intestine. This is just the contrary of current teaching according to which morphin belongs to the pyridin-phenanthrene group and stimulates excised intestine, increasing especially its tone. Trendelenburg regards the functional activity of guinea pig intestine to be more like that of human intestine. He also could not confirm the potentiation of narcotin on morphin in the mixture called, "narcophin," according to the claims of Straub for intestine and those of Macht for other functions (psychic, coronary artery, etc).

This brief survey indicates that the current classification of the opium alkaloids into the two groups mentioned above and the claims of potentiation, etc., are not necessarily as broadly significant nor as fundamental as they appeared to be originally. Therefore, a study of their effects will bear repetition, at least in another direction, for confirmation or denial as the case may be. Some allowances, of course, must be made for differences in functional activity of, and the dosage or concentrations of the drug necessary for, cardiac muscle. The results with the alkaloids on cardiac muscle may now be described. Comment will also be made on the seat of depression of the benzyloquinolin alkaloids on the heart.

II. METHODS

Straub's method of perfusion was used with hearts of frogs and also some turtle hearts. A long narrow glass-cannula was inserted through the aorta into the cavity of the ventricle and securely tied. The remaining vessels were then ligated and the excised heart was transferred to a moist chamber supplied with a constant stream of oxygen, a part of which was conducted at the same time into the ventricular cannula, containing Ringer's solution. The drugs in different concentrations in Ringer's solution were introduced directly into the cannula and washed out by means of suction and a fine pipette. A light lever attached to the apex of the ventricle by means of a fine wire clip and silk thread passing through an opening in the bottom of the chamber were used to obtain records of the contractions on a slow moving kymograph.

The remaining hearts of turtles were perfused in the usual way by placing cannulas into a vein and into an aorta. The vein cannula was connected to two bulbs by tubing with a by-pass for convenient removal of the drug solution from one of the bulbs. The other bulb was used for plain Ringer's solution. The remaining vessels were ligated and the perfusion continued with the heart in situ recording the movements on a slow moving kymograph.

Both atropinized and untreated hearts were used. The experiments were about equally divided. The results were qualitatively the same though some times different quantitatively. This was especially true of the stimulant agents. That is, the augmentor effects of these agents on some atropinized hearts were not quite as pronounced as those on the untreated hearts. This is attributed to the depressant action of atropin on the cardiac muscle. The concentration of 0.1 per cent atropin was used. On the other hand, the effects of depressors on atropinized hearts were somewhat more pronounced, if anything, assisted no doubt by the additional depressant influences of atropin. It is obvious, therefore, that the agents exerted their effects on the heart independently of parasympathetic nerve connections.

III. RESULTS WITH INDIVIDUAL ALKALOIDS

The results on the hearts of the frogs and turtles were so much alike that they are described together. In order to conserve space as much as possible all controls made with Ringer's solution are not reproduced. Only the necessary illustrative tracings have been included in the paper.

Morphin. The results of fifty-one applications with concentrations of morphin hydrochloride, ranging from 0.01 to 0.2 per cent consisted of a prompt and sometimes marked increase

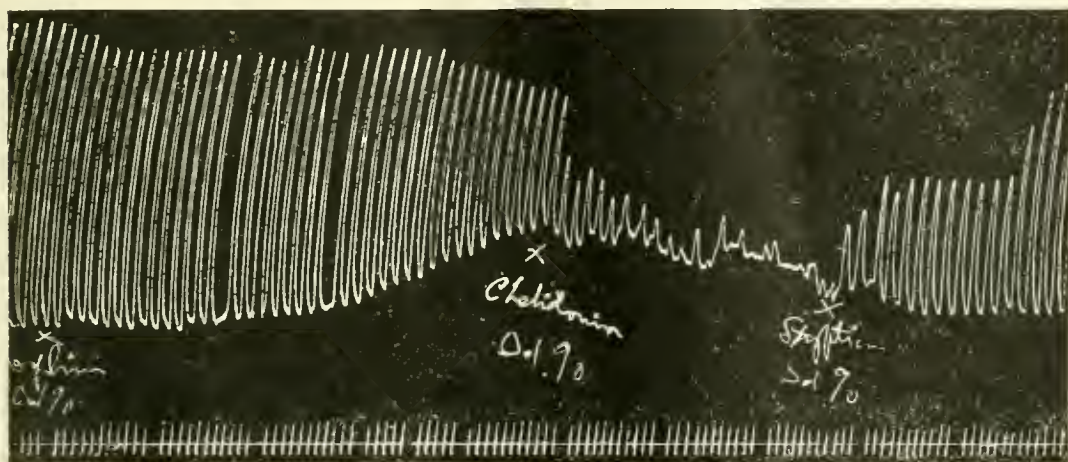


FIG. 1. EFFECTS OF MORPHIN ON UNTREATED TURTLE HEART, OF CHELIDONIN AFTER MORPHIN AND OF COTARNIN HYDROCHLORID (STYPTICIN) AFTER CHELIDONIN ON THE SAME HEART

Time, each stroke = five seconds. All tracings read from left to right. Up-stroke means systole and downstroke, diastole.

in tonus with a temporary increase in rate followed by a prolonged slowing and marked diminution in amplitude of contractions. Removal of the morphin by washing with plain Ringer's solution gradually, though not always, restored the rate and amplitude of contractions. As a rule, the tone remained somewhat elevated, but could be promptly lowered by antagonists. This is illustrated by figure 1.

Higher concentrations (1 per cent and 4 per cent) caused complete inhibition of contractions with recovery by prompt

washing with Ringer's solution or the rise of antagonists. The tonus in the beginning is also raised by these concentrations.

The effects of low concentrations of morphin on cardiac muscle are interpreted as stimulation, and agree closely with those on smooth muscles of various excised organs (uterus, ureter, intestine, etc.) when studied in the usual manner. The chief and primary factor in the stimulation is the marked increase in tonus, the increase in rate being secondary, while the amplitude is diminished due to the marked increase in tonus. The result is a marked systolic tendency of the heart with corresponding diminution in output.

While the conditions in the mammalian heart are different, yet it can be readily seen that morphin even in large therapeutic doses would not injure the heart seriously by direct action; nor could it be objected to in circulatory collapse of cardiac origin.

The effects on tonus observed in these experiments are confirmative of those observed by Snyder and Andrus (5) on the perfused atropinized heart of turtle. From observations on the rate and measurements of outflow of atropinized frog's heart, Worth Hale concluded that low concentrations of morphin possessed a stimulant action, though no reference is made to the effect on tonus. High concentrations were found by Hale to cause depression. However, it is difficult to see how the method used by Hale could give an adequate idea of tonus action (the main factor in the stimulation), without a graphic record. That is, changes in output can be easily compensated by reciprocal changes in amplitude and pulse rate. The same output in a given time, although increased per beat, could be obtained with a slowed and relaxed heart as with the same heart exhibiting a greater systolic tendency (stimulation) and rapid pulse. The graphic records which were obtained in figures 1 and 6 indicate a slight increase or no change in rate with a marked systolic tendency of the morphinized hearts owing to the marked increase in tonus. Consequently the output must be diminished, although the organs were markedly stimulated. This interpretation would be improbable from a knowledge of the changes in rate and output only. In my experiments, the only

effect of low and high concentrations of morphin on tonus was invariably an increase. To this extent the results of Snyder and Andrus (5) and myself differ from those of Hale and some of the older observers.

Papaverin. This benzyloquinolin alkaloid has been sufficiently previously studied by others (Hale (1); Macht (2)) on cardiac muscle, and the results of the two experiments made fully confirm their results. The effects consisted of a prompt lowering of tonus with marked slowing of rate and reduction of amplitude with low and medium concentrations; complete stoppage and paralysis with high concentrations.

This depression of cardiac muscle produced by papaverin agrees with its action on smooth muscle in various regions. The mechanism of the cardiac tonus depression is attributed by Snyder and Andrus (5) to depression of the smooth muscle of the walls of the heart (turtle). This explanation does not appear convincing and will be referred to again later in the paper.

Chelidonin. This alkaloid is closely related to papaverin chemically and also pharmacologically as far as smooth muscle and some other effects are concerned (6). Because of this and its chemical composition it is classed with the benzyloquinolin group. However, the effects on heart directly have not been previously studied by the method here used. Hale (1) studied the effects of chelidonin on rate and output of perfused frog heart, and found both to be decreased.

The results of twenty-five applications to cardiac muscle in different ways, and using different concentrations (0.01 per cent to 5 per cent) show that chelidonin behaves precisely the same way as papaverin. The tonus was invariably lowered, the rate slowed and the amplitude diminished. Removal of the drug by washing with Ringer's solution resulted in prompt recovery. Paralysis was produced by a 5 per cent concentration. It seemed that higher concentrations were required to cause paralysis than with papaverin. The results are illustrated by figures 1, 2 and 6.

These depressant effects on cardiac muscle agree with those on smooth muscle in various regions, and are exactly opposed to

those of morphin as far as changes in tonus and initial rate are concerned, and when acting under the same conditions. The depressant effects of morphin on intestine reported by Trendelenburg are obtained with small doses and a different method, precluding a strict comparison with the results referred to here which were obtained by ordinary methods of studying strips of excised organs. It will be recalled that the lasting effect of morphin on rate is to slow the heart and to diminish the amplitude of contraction. To this extent the late effects of morphin on rate and amplitude are similar to those of chelidonin, but not necessarily synergistic. That is, the shortening of amplitude by

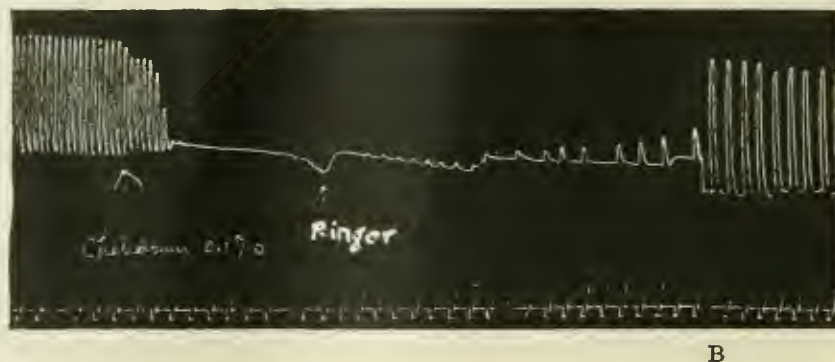


FIG. 2. A. EFFECTS OF CHELIDONIN ON FROG'S HEART AND GRADUAL RECOVERY AFTER WASHING WITH RINGER'S SOLUTION. B. RECOVERY AT THE END OF ONE AND ONE-HALF HOURS AFTER APPLICATION OF RINGER'S SOLUTION

Time, strokes at intervals of five seconds

morphin can not be attributed to depression but rather to the marked tonus effect, which prevents relaxation. On the other hand, the tonus is characteristically lowered by the benzyloquinolin group (chelidonin and papaverin).

Cotarnin salts. According to the results of Macht (2) on excised ureter, the cotarnin salts stimulate the contractions (especially the tone of smooth muscle) and, therefore, belong with morphin in the pyridin-phenanthrene group. In previous experiments (7) on perfused blood vessels of the frog and uterus, cotarnin was shown to cause relaxation, presumably due to depression of smooth muscle. The results of forty-two applications of cotarnin

hydrochloride (stypticin) in different concentrations (0.01 per cent to 0.1 per cent) to cardiac muscle indicate a moderate and temporary increase in tone followed by a fall. The pulse rate was increased or unchanged, while the amplitude was always markedly increased. The amplitude continued to increase while the tone continued to fall, thereby, increasing the output of the heart. In some of the unatropinized hearts of turtles cotarnin hydrochlorid moderately decreased the tone from the start, the effects on amplitude and rate being the same as in the remaining untreated and atropinized hearts. Washing with Ringer's solution promptly brought recovery. In very high

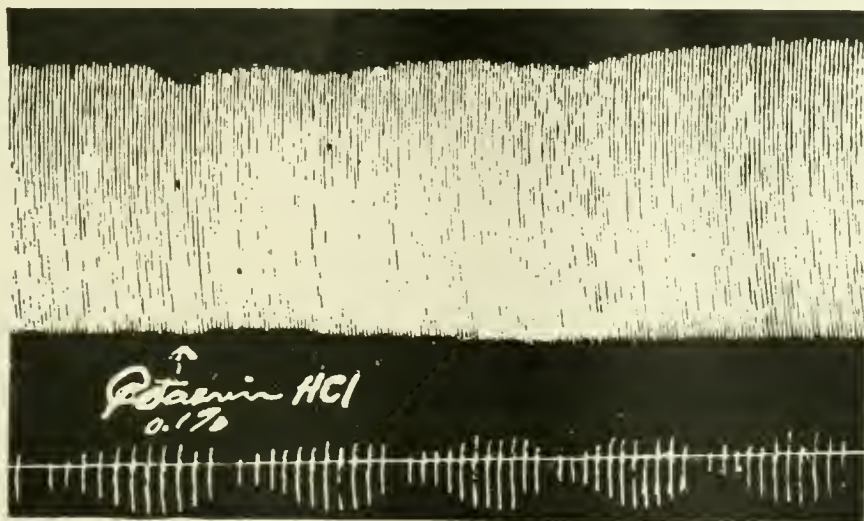


FIG. 3. EFFECTS OF COTARNIN HYDROCHLORID ON ATROPINIZED FROG'S HEART

Time, each stroke = five seconds

concentrations (2 per cent) cotarnin was also found to inhibit the heart completely. Cotarnin phthalate (14 applications) lowered the tone and reduced the rate and amplitude. Figures 1, 3, 6 and 8 illustrate the effects that were obtained under various conditions.

The results with the two cotarnin salts were not exactly uniform, and, since the uniformity and number of experiments were greater with cotarnin hydrochlorid, the following discussion is limited to this salt. The effects resembled in part those of morphin on the tone of cardiac and smooth muscle. While morphin

diminishes the amplitude, the rate being temporarily increased and then slowed, cotarnin, as a rule, increased the amplitude and rate. In both cases, the net result is equivalent to stimulation, the morphin stimulation being expressed chiefly by the tonus effect, while the cotarnin stimulated by increased cardiac output. To this extent it may be said that the drugs are synergistic and agree in their effects on cardiac and smooth muscles.

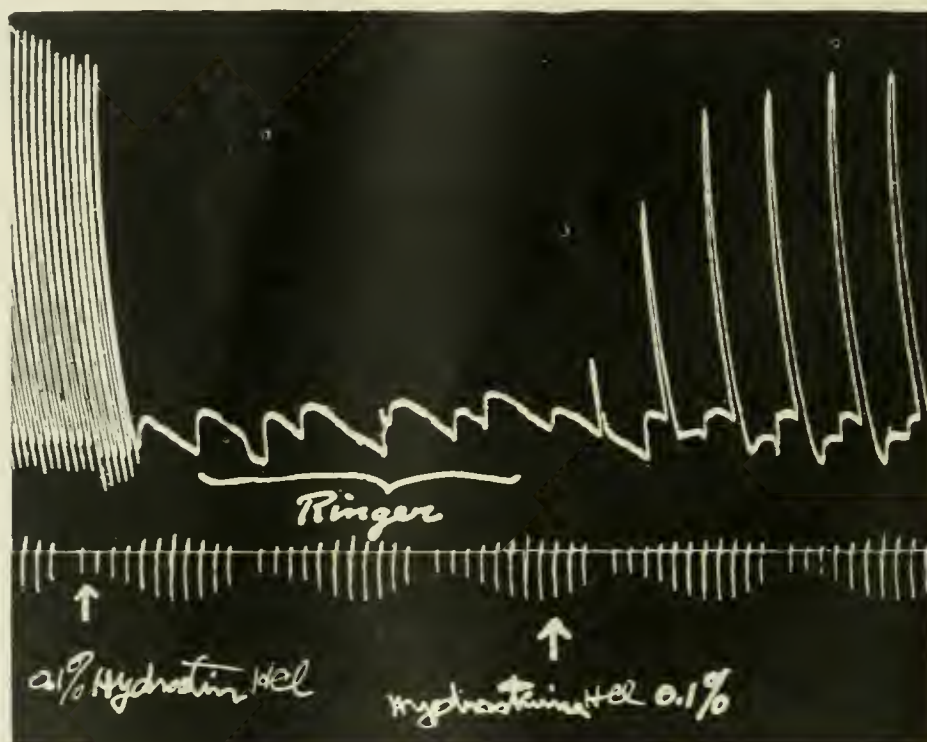


FIG. 4. EFFECTS OF HYDRASTININ HYDROCHLORID AFTER HYDRASTIN HYDROCHLORID ON ATROPINIZED FROG'S HEART

Time, each stroke = five seconds

Strictly speaking, however, this is not true when all effects are taken into consideration. In fact, cotarnin, partakes of the effects of both the phenanthrene and isoquinolin alkaloids. This will be discussed again in the section on antagonism.

Hydrastinin. As far as ureteral effects are concerned, this drug belongs with morphin in the pyridin-phenanthrene group. However, the results of fifteen applications show that the effects on cardiac muscle are different. In the majority of experiments

the tone was unchanged or slightly increased, the pulse rate slowed and the amplitude was definitely increased, resembling in part (tone and amplitude) the effects of cotarnin. This is illustrated by figure 4. Here again, there is a lack of agreement between smooth and cardiac muscle effects. The systemic effect of hydrastinin on the blood vessels is said to be constrict-

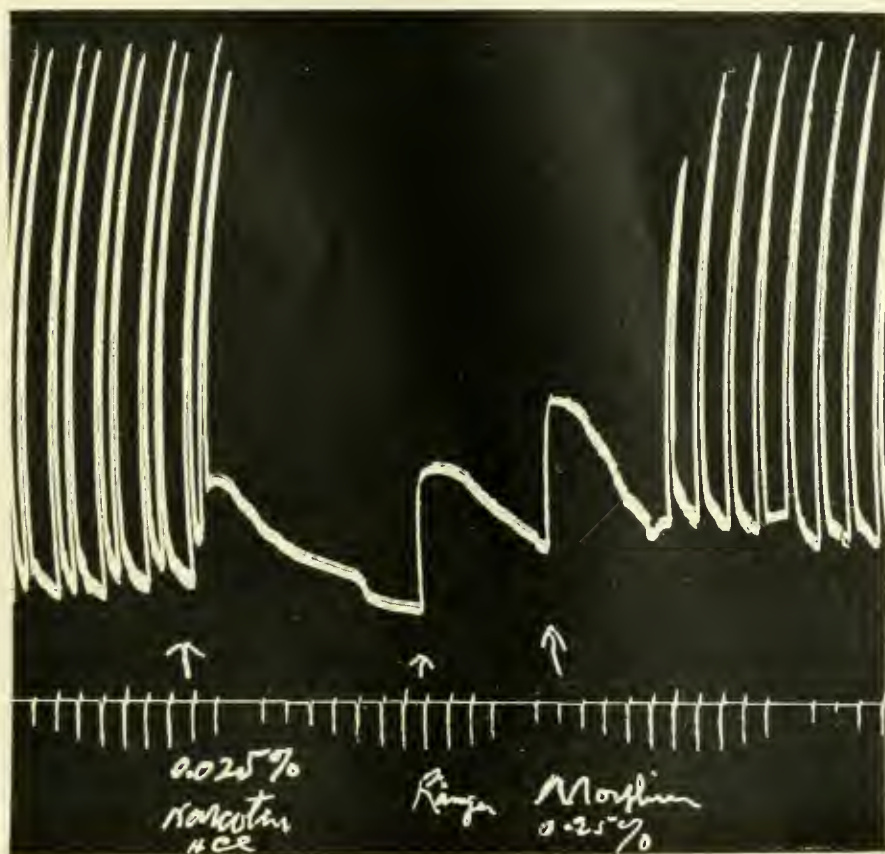


FIG. 5. EFFECTS OF NARCOTIN HYDROCHLORID ON ATROPINIZED FROG'S HEART; ANTAGONISM BY MORPHIN HYDROCHLORID

Time, each stroke = five seconds

tion, and this, of course, need not be on the smooth muscle. Systemically hydrastinin slows the pulse. This harmonizes with my results on the perfused heart, although the mechanism of action in the two cases may be different.

Hydrastin. Chemically this drug is a close relative of narcotin and papaverin, and, therefore, belongs to the benzyloisoquinolin group. Macht showed that it possesses a papaverin-like action

on the smooth muscle of the ureter. As illustrated by figures 4, 7 and 8, the results of my experiments on cardiac muscle agree with those on the ureter. That is, hydrastin in low concentrations invariably lowers the tone, slows the pulse rate and temporarily reduces the amplitude of contractions, resembling the actions of papaverin and chelidonin. In high concentration cardiac arrest in the diastolic position is obtained. The systemic vasoconstriction, resulting in a rise of blood pressure, may not be due necessarily to constriction of smooth muscle. In general, it can be said that hydrastin acts like a benzyloquinolin derivative on different muscles.

Narcotin. The results of two applications fully confirm the results of Hale on cardiac muscle, and those of Macht on the smooth muscle of the ureter. That is, this alkaloid is a very efficient depressant of tonus and the rate and amplitude of cardiac contractions in low concentrations, and produces complete arrest and paralysis of the heart in high concentrations. Figure 5 illustrates the effects of 0.025 per cent narcotin.

IV. ANTAGONISTIC AND SYNERGISTIC ACTIONS

Morphin and chelidonin. According to the descriptions above the effects of one should be removed by the other of these alkaloids, providing paralysis of the heart is not complete. This was found to be the case as illustrated by figures 1 and 6. These results agree with those on smooth muscle previously reported. It was also shown that chelidonin can antagonize the constrictor action of morphin on the perfused blood vessels of the frog (Läwen-Trendelenburg method).

Morphin and cotarnin. Figure 6 illustrates that an equal concentration (0.1 per cent) of cotarnin can oppose the action of morphin. The application of cotarnin hydrochlorid to a morphinized heart showing the usual effects caused the opposite, namely, lowered the tone and increased the rate and amplitude of contractions. The application of morphin to a cotarnin heart showing the usual effects, also produced the opposite, namely, increase in tone and reduction of rate and amplitude.

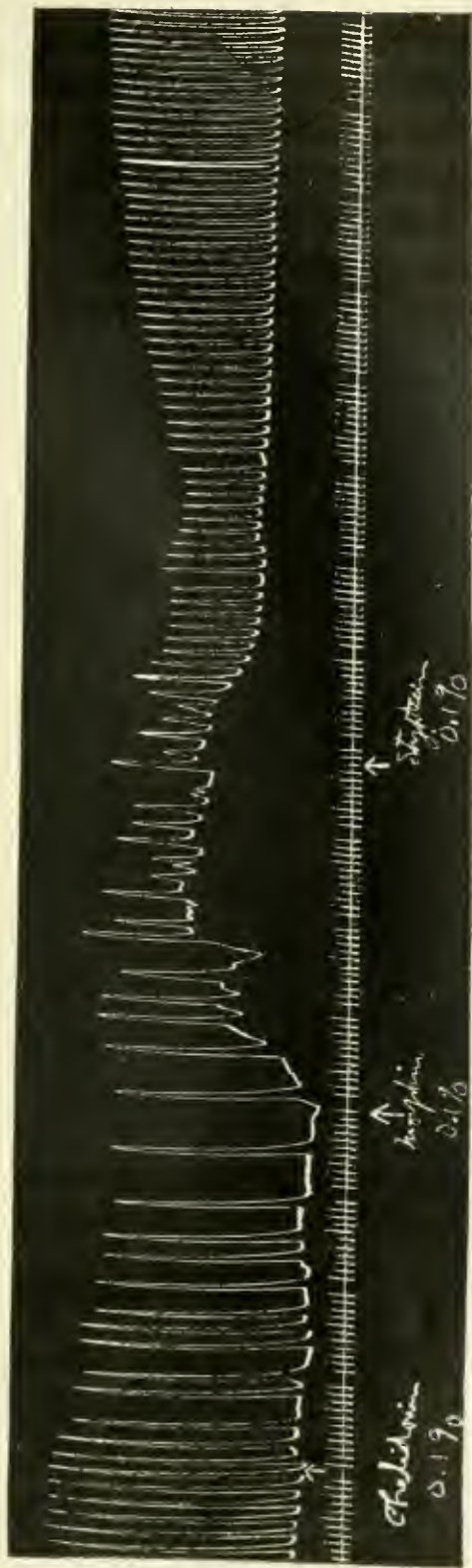


FIG. 6. EFFECTS OF CHELIDONIN SULPHATE ON UNTREATED TURTLE'S HEART, OF MORPHIN HYDROCHLORID AFTER CHELIDONIN AND OF COTARNIN HYDROCHLORID (STYPTICIN) AFTER MORPHIN

Time, each stroke = five seconds

Morphin was also found to oppose the action of cotarnin phthalate (styptol) on the heart. The antagonism between these alkaloids was also shown in mixtures of the two. In such mixtures cotarnin can prevent the full development of morphin action on cardiac muscle. That is, in a mixture of cotarnin hydrochlorid and morphin hydrochlorid, containing equal concentrations of each, the frog's heart showed only a slight, though definite increase in tonus (predominant morphin action), and an increase in rate and amplitude (predominant cotarnin action). The application of morphin alone to the same heart after the removal of the mixture by washing with Ringer's solution produced only a typical morphin action, namely, marked increase in tone, and reduction in amplitude with no change in rate.

According to these results, cotarnin can not be regarded as a synergist of morphin. In fact, cotarnin fits neither into the pyridin-phenanthrene group nor into the benzyliisoquinolin group of alkaloids accurately, but rather partakes of the physiological qualities of both. The results on cardiac muscle do not agree with those (all effects) on the smooth muscle of the ureter. They agree with those on perfused blood vessels. Its chemical derivation (from narcotin) would place it theoretically into the benzyliisoquinolin group. However, the results of this report indicate the imperfection of the classification. They indicate further that the differences in action that may appear on a given organ or function are not so fundamentally or generally applicable as they may seem.

Morphin and hydrastin. Morphin would be expected to antagonize the depressant effects of hydrastin. The results of seven applications of morphin to different hearts treated with hydrastin confirmed this. The tonus was raised and the rate and amplitude were increased, resulting in complete and prompt recovery as a rule. This is illustrated by figure 7.

Morphin and hydrastinin. The results of five applications of hydrastinin to different morphinized hearts indicated almost complete recovery from the effects of the morphin. That is, the tonus was lowered and amplitude and rate were increased. This is shown in figure 4.

Morphin and narcotin. In the two experiments that were made these alkaloids were shown to exert exactly opposite effects on cardiac muscle. Morphin augmented the tone, rate and amplitude of the hearts depressed by narcotin, and narcotin depressed and completely inhibited the contractions of morphinized hearts. Figure 5 illustrates this partly.

Narcophin. This is a mixture of morphin and narcotin. A concentration of 0.01 per cent was found to depress and 0.1 per cent stopped the heart. Both experiments give evidence of

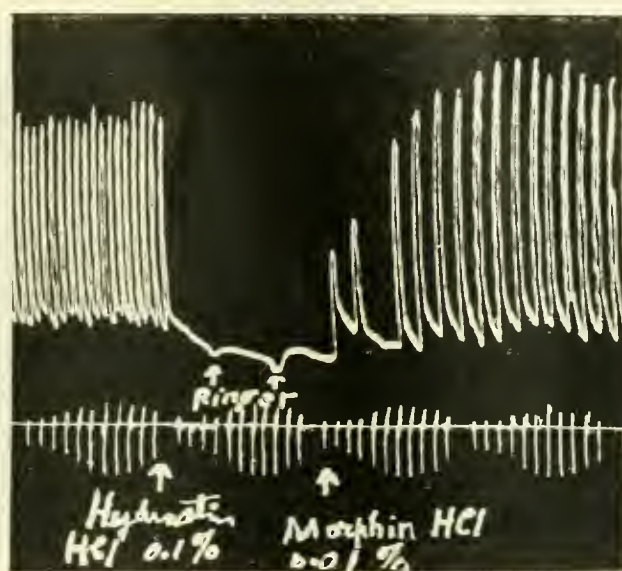


FIG. 7. EFFECTS OF MORPHIN HYDROCHLORID AFTER HYDRASTIN HYDROCHLORID ON ATROPINIZED FROG'S HEART

Time, each stroke = five seconds

the preponderating influence of the depressant constituent, narcotin. This is contrary to the claims made for the product, namely, of potentiation of the morphin constituent. Although this potentiation was claimed largely for smooth muscle (intestine, etc.), nevertheless cardiac muscle should not be an exception. This potentiation has not been sustained by the recent work of Trendelenburg with a new method of studying intestinal peristalsis and referred to in the fore part of the paper.

Cotarnin and hydrastin and also chelidonin. From what has been said, cotarnin would be expected to antagonize the actions

of hydrastin and chelidonin on cardiac muscle. Figure 8 illustrates that cotarnin causes some increase in the tone after hydrastin and more marked increase in rate and amplitude of contractions. Figure 1 shows that cotarnin does practically the same after chelidonin. That is, there is an increase in the rate and amplitude with maintenance of tone. The antagonism as to effects is as perfect as could be expected under the conditions. The results are adduced as further evidence that hydrastin and cotarnin, while closely related chemically to their parent

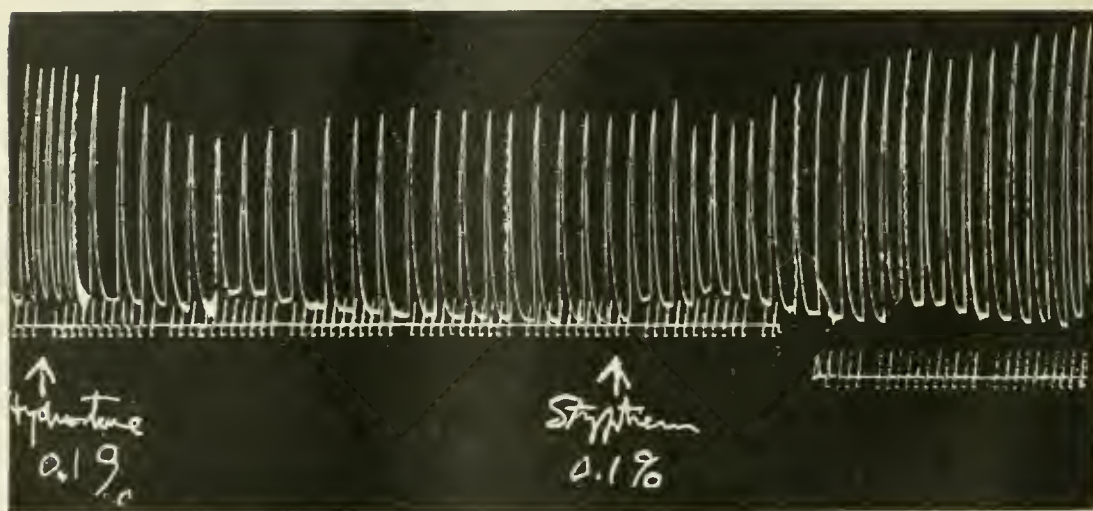


FIG. 8. EFFECTS OF HYDRASTIN ON TURTLE'S HEART AND ANTAGONISM BY COTARNIN HYDROCHLORID (STYPTICIN)

Time, each stroke = five seconds

substance, narcotin, are not necessarily similar in their physiological effects.

Cotarnin and hydrastinin. A partial synergistic action would be expected from these alkaloids. The results of five applications of cotarnin to hearts treated with hydrastinin showed no change or some diminution in tone and an increase in rate and amplitude. The tendency to recovery seemed to be greater, although qualitatively the effects were practically the same except for the pulse rate which is generally slowed by hydrastinin alone.

Hydrastinin and hydrastin. The lowered tone of a frog's heart after hydrastin was not promptly restored by washing with Ringer's fluid, but the application of hydrastinin raised the tone and restored the amplitude of contractions, the rate remaining much slower than originally, as might be expected. Figure 4 illustrates these effects.

V. SEAT OF DEPRESSANT ACTION OF THE BENZYLISOQUINOLIN GROUP ON THE HEART

It is not the object here to discuss whether the benzylisoquinolin group as a whole or in part is responsible for the depression produced by its representative members (chelidonin and papaverin). The weight of evidence to date is in favor of the benzyl constituent. If this is true, undoubtedly it applies to chelidonin as well as to papaverin, but by no means, of course, to some of the alkaloids of this paper whose actions nevertheless resembled those of papaverin and chelidonin. The object of consideration here is the site of action as it pertains to the element in the heart on which these agents act to produce the depression, especially depression of tonus.

In a recent paper by Snyder and Andrus (5) along this line, but with a different aim, remarkable conclusions are reached concerning the depressant action of papaverin (also benzylalcohol) on the terrapin heart. According to these authors these agents act specifically on smooth muscle, and since terrapin heart contains smooth muscle besides the regular cross-striated cardiac type, they argue that the depressant action of these drugs on tonus is due to their action on the smooth muscle portion of the heart walls. This explanation might be satisfactory if the alleged specificity of these agents on, and the presence of smooth muscle in, the heart could be accepted without question. However, the specificity does not exist and the presence of smooth muscle in the wall of heart has not been conclusively demonstrated. Therefore, the premises on which Snyder and Andrus based their problem are false.

As to the alleged specificity of papaverin for smooth muscle, it is known that this tissue is sensitive to and acted upon by papaverin independently of nerve connections. However, it has been shown by Pal (8) that papaverin and by H. H. Meyer (9) that chelidonin also depress skeletal muscle, and by Macht and Weiner (10), Macht and Fischer (11) and Pick and Wasicky (12) that papaverin depresses other structures free from muscle, namely, paramacia, trypanosomes, colpidia and amebas. Therefore, the claims of specificity of action of papaverin on smooth muscle in the true sense are not justified. The differences in action on smooth as compared with other kinds of muscles are probably of a quantitative nature and this is quite sufficient to account for the prompt response of smooth muscle.

I have shown also with chelidonin, instead of papaverin, as a representative of the benzyloquinolin group, that frog's gastrocnemii are depressed by this agent. The muscles were immersed in chelidonin sulphate of different concentrations, using Ringer's solution as control, and testing their irritability by determination of the minimal effective stimuli at short intervals. These results showed a gradual depression of the skeletal muscle with low, and death with high concentrations, while the controls in Ringer's solution showed practically no changes worthy of mention. This is confirmative of the results obtained by H. H. Meyer (9) with chelidonin on frog's muscle directly and by nerve stimulation. Figure 9 illustrates the depressant action on a frog's gastrocnemius immersed in Ringer's solution containing chelidonin and stimulated electrically through fine wires imbedded in the muscle substance. This leaves no doubt as to the depressing properties for muscles of different origin by representative benzyloquinolin members. These experimental data are sufficiently conclusive against the assumption of specificity of papaverin for smooth muscle. However, this does not adequately dispose of the other claim by Snyder and Andrus, namely, that smooth muscle exists in the walls of the heart. This is, of course, intimately connected with the main proposition under consideration, namely, the determination of the site of action of papaverin (and also, of course, the cause of

the tonus oscillations in turtle heart, although that is not the special concern of this paper).

According to Snyder and Andrus (5) on page 1 of their paper, "The tonus changes it will be remembered are most marked in the auricular walls where the smooth fibers are most abundant." On page 9 they say "It is also known that smooth fibers are present in the wall of the ventricle" Snyder and Andrus base their statements on a paper by Rosenzweig (13) on tonus variations in turtle heart. Rosenzweig's paper contains a brief note on the presence of smooth-muscle-like elements in

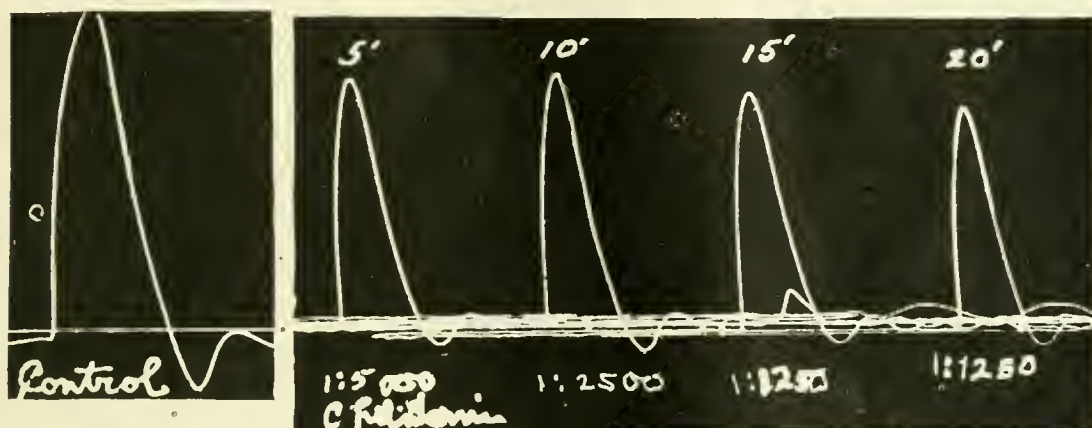


FIG. 9. EFFECTS OF DIFFERENT CONCENTRATIONS OF CHELIDONIN SULPHATE IN RINGER'S SOLUTION ON FROG'S GASTROCNEMIUS MUSCLE

Single shock stimulations at end of 5, 10, 15 and 20 minutes after immersion. Fall of tone is indicated by progressively lower position of muscle lever.

teased preparations and sections of turtle auricles. Apparently Snyder and Andrus attach more significance to the rôle and presence of smooth muscle in Rosenzweig's work than even Rosenzweig himself felt justified in doing. In attempting to explain the cause of the tonus oscillations (isochronic contractions) of turtle heart, Rosenzweig proposed a hypothesis on pages 206 and 207 of his paper. The chief elements in this hypothesis are that these oscillations are localized in special muscle cells which are enclosed in the ordinary cardiac tissues, and that the rhythmic contractions occur only as a result of maximal stimuli such as sudden hemorrhage, death, etc. Rosen-

zweig adds that such a hypothesis has no value without histological facts, which he proceeded to obtain on teased preparations of turtle auricles treated with fuming nitric acid and 33 per cent potassium hydroxide. It is obvious that this is rather heroic treatment, and that the preparations probably contained artefacts. Nevertheless, in such preparations many cells without cross striations were found and could not be differentiated from smooth muscle cells. However, this was not satisfactory and Rosenzweig stained sections of the auricles. In these sections spindle cells with ovoid nuclei, but without striations, were present as a layer in the *endocardium*. In the closing sentence of his paper, Rosenzweig states that his hasty preliminary examinations are not decisive. Incidentally it may be mentioned that the suggestion that other elements besides cardiac muscle play a rôle in the tonus oscillations was first made by their discoverer, Fano (14). The more fervent claims of Bottazzi (15) as to the rôle of smooth muscle in these tonus oscillations were even less supported by facts than the hypothesis of Rosenzweig. It should be added also that the presence of the tonus oscillations in turtle heart observed by Fano were confirmed by Rosenzweig with certain reservations. Rosenzweig regards this phenomenon as an abnormality and inconstant in appearance for both the auricle and ventricle. He also demonstrated their presence in the ventricle, which Fano originally denied.

This brief résumé of the histological evidences for the presence of smooth muscle in turtle heart justifies the following conclusions, which invalidate the claims of Snyder and Andrus.

(1) The presence of smooth muscle in the walls of turtle heart, i.e., the myocardial mantle (auricles and ventricles), has not been demonstrated at all. (2) If any smooth muscle exists, it is in or on the endocardial layer. (3) Therefore, this is unlikely to play any rôle whatsoever in the functional activity of the heart, such as would be reflected in variations of the tonus, rate and amplitude of the contractions. (4) Tonus oscillations (isochronic contractions) of turtle heart have not been as yet properly explained, and certainly not by the presence of smooth muscle. (5) Adequate smooth muscle, which would be of

importance in the functional activity (contractions) of the walls of the heart of any species whatsoever, still remains to be demonstrated, excluding here, of course, the smooth muscle of the blood vessels. Pathological and normal hearts of man have been shown by Van der Stricht and Todd (14), to contain smooth muscle cells. However, here again the strands of smooth muscle are present in the endocardium only. Professor Todd assures me that smooth muscle does not occur in the myocardial wall of the human heart, which is the only one that has been sufficiently studied. This is in general agreement with Rosenzweig's statement concerning his sections of turtle heart, and fails to give support to or justify the claims of Snyder and Andrus.

Consequently, there are no grounds for considering the depressant action exerted by papaverin (and also chelidonin) on the heart as being due to the smooth muscle element. As a matter of fact, the representatives of the benzyloquinolin group (chelidonin and papaverin) act on all varieties of muscle cells in the same sense. They produce direct effects, which differ chiefly quantitatively, resulting in depression or paralysis. Therefore, there is every reason to believe that these alkaloids can alter the functional activity of (depress) cardiac muscle cells just as they do those of smooth and skeletal muscle cells. It is suggested that sarcoplasm, whether in cardiac, skeletal or smooth muscle cells, independently of nerve connections, and protoplasm generally (unicellular organisms, etc.) are depressed by the benzyloquinolin alkaloids (papaverin and chelidonin). The antagonistic effects of barium on all muscles may be adduced as further evidence in support of the suggested action of the benzyloquinolin alkaloids (chelidonin and papaverin).

VI. DISCUSSION

The effects of the individual agents have been sufficiently discussed in the text and need not be repeated here. It will suffice to indicate the general tendencies in the correlation of the cardiac actions and chemical structure and origin of the different agents, and comparison of actions on different muscles. This

will be assisted by tabulations of the results. Table 1 contains a summary of the principal effects of the majority results with the individual agents and antagonists on untreated and atropinized hearts together with their interpretations. Table 2 con-

TABLE 1
Summary of effects on untreated and atropinized hearts (turtle and frog)

DRUG OR TREATMENT USED	TONE	RATE	AMPLI- TUDE	INTERPRETATION
Morphin	+	+ sl.	—	Stimulation
Papaverin	—	—	—	Depression
Chelidonin	—	—	—	Depression
Cotarnin	+	+	+	Stimulation (variable)
Hydrastinin	±	+	+	Stimulation
Hydrastin	—	—	—	Depression
Narcotin	—	—	—	Depression
Narcophin	—	—	—	Depression
Morphin after hydrastin	+	+	+	Stimulation
Morphin after cotarnin	+	—	—	Stimulation
Morphin after narcotin	+	+	+	Stimulation
Morphin after chelidonin	+	+	+	Stimulation
Chelidonin after morphin	—	—	—	Depression
Cotarnin after morphin	—	+	+	Mostly stimulation
Cotarnin after hydrastinin	—	+	+	Mostly stimulation
Cotarnin after hydrastin	+	+	+	Stimulation after low concentration
Cotarnin after hydrastin	—	—	—	Ineffective after high concentration
Cotarnin after chelidonin	+	+	+	Stimulation
Narcotin after morphin	—	—	—	Depression
Hydrastinin after morphin	—	+	+	Mostly recovery
Hydrastinin after hydrastin	±	±	±	Restoration with low, ineffective with high concentration
Hydrastin after hydrastinin	—	—	—	Depression

* The plus sign (+) means increase, the negative sign (—), decrease.

tains a summary of the results on cardiac muscle of this report and on smooth and skeletal muscles and unicellular organisms culled from various sources in the literature.

The summary in table 2 shows that the effects of papaverin, chelidonin and narcotin on cardiac, smooth and skeletal muscles

and unicellular organisms agree almost without exception and consist of depression. The remaining agents lack this uniformity of action. Except for its variable action on smooth muscle, hydrastin could be classed with the depressants. On the other hand, morphin is a stimulant or without effect, while hydrastinin and cotarnin are variable, being stimulant or depressant or

TABLE 2

Summary of effect of the different alkaloids on different kinds of muscles and unicellular organisms

AGENT	CARDIAC MUSCLE	SMOOTH MUSCLE	SKELETAL MUSCLE	UNICELLULAR ORGANISMS, INCLUDING PARASITES
Papaverin . . .	Depresses	Depresses	Depresses	Very toxic*
Chelidonin . .	Depresses	Depresses	Depresses	
Narcotin	Depresses	Depresses†	Depresses	Toxic
Hydrastin . . .	Depresses	Variable‡	Depresses	Toxic
Hydrastinin . .	Stimulates	Variable§		Innocuous
Cotarnin	Moderate stimulation of untreated; moderate depression of stimulated	Variable**		Innocuous
Morphin	Stimulates	Stimulates††	No effect	Moderate toxicity

* Paramecia, colpidia, ameba and trypanosomes; for remaining agents of this column, paramecia and trypanosomes.

† Bronchial stimulation of pithed dogs (17).

‡ Excised uterus stimulated, ureter depressed.

§ Excised uterus and artery stimulated; no action on intestine and bladder; ureter depressed.

** Excised ureter, uterus and intestine stimulated; bronchi and bladder not constricted (18); artery depressed.

†† Excised intestine of guinea-pig depressed; intact human intestine inhibited.

inactive. The cardiac effects of papaverin, chelidonin and narcotin agree with those reported by Hale, but differ as to morphin on tone. The effects of morphin on turtle and frog hearts observed by me agree with those of Snyder and Andrus on turtle heart. Hale concluded that cardiac stimulation by morphin occurred only with very low concentrations of the drug. In my work both low and high concentrations were characterized by initial stimulation, although it is true that very high concen-

trations (2 per cent and 4 per cent) caused complete inhibition of the contractions. This will hold true of all of the agents here reported and the summary in table 2 refers to low and moderate concentrations and less than those causing inhibition. Such high concentrations, of course, could not occur in the circulation, and, therefore, their practical significance is distinctly limited and unimportant.

Since the effects of the different alkaloids studied on atropinized and untreated hearts were the same it is obvious that the depressants exerted their action directly on cardiac muscle independently of the parasympathetic endings.

Attempts to correlate the chemical structure of the agents studied with their cardiac effects are beset with greater difficulties. A reproduction of the structural formulae here would add very little to a proper understanding of this. It will suffice to indicate a number of the inconsistencies, which render a strict classification into the pyridin-phenanthrene or muscle stimulant, and benzyloquinolin or muscle depressant group of limited value. For instance, cotarnin, hydrastin, papaverin, chelidonin, narcotin and hydrastinin are closely related to each other in chemical structure and composition or by derivation, yet it is seen in table 2 that cotarnin and hydrastinin possess opposite muscular actions to those of the remaining alkaloids. It has been suggested that this might be accounted for by the presence or absence of certain chemical groups, but this is doubted. For, morphin, which is quite different in chemical structure and composition from hydrastinin and cotarnin, nevertheless possesses similar actions on the same and different muscles. The same is true of narcotin, which is chemically different from morphin, yet the action on some smooth muscles is exactly the same, and both alkaloids are good antagonists on turtle's heart. The difficulties of making strict correlations between chemical structure and pharmacological action are therefore obvious, even where the effects agree on several different, though related, morphological structures. It would seem that compounds or agents with very close or nearly identical chemical structure should possess nearly identical pharmacological actions.

This is true of two such alkaloids as papaverin and chelidonin, perhaps also of narcotin and hydrastin, and hydrastinin and cotarnin. The results of this paper may serve to emphasize the desirability of conservatism at a time when the tendency to much generalization and speculation along these lines is rife.

VII. CONCLUSIONS

1. Using the perfused amphibian heart as test object the effects of a number of representatives of the benzyloquinolin group of alkaloids were compared with the action of morphin.

2. Morphin (pyridin-phenanthrene) augmented the tone, temporarily increased but later slowed the rate and shortened the amplitude, while papaverin, chelidonin, hydrastin and narcotin (benzyloquinolin) lowered the tone, slowed the rate and reduced or abolished the amplitude of contractions of the perfused hearts. These effects of papaverin, chelidonin and narcotin agree with those on smooth muscle in various regions and skeletal muscle independent of the innervation, while hydrastin is variable.

3. Cotarnin hydrochlorid (stypitacin) and hydrastinin, which belong chemically to the benzyloquinolin group, possessed actions similar to morphin (pyridin-phenanthrene group), the general effects on rate, tonus and amplitude of contractions being equivalent to stimulation, although individual variation was encountered. These effects do not always agree with their effects on the smooth muscle of various organs.

4. Antagonistic effects on cardiac muscle were produced in mixtures and by independent applications of the following alkaloids; morphin and chelidonin, morphin and cotarnin salts, morphin and narcotin, cotarnin and hydrastin and cotarnin and chelidonin and hydrastinin and hydrastin.

5. The results obtained indicate various difficulties involved in the correlations of chemical structure and pharmacological actions and the unreliability of classifications derived from limited data. However, a remarkably close agreement in the

effects of papaverin, chelidonin and narcotin on different muscles, namely, cardiac, smooth and skeletal, was found to exist.

6. Therefore, the alleged specificity of papaverin for smooth muscle is not sustained by the results of this investigation. This also applies to chelidonin.

7. There is no satisfactory proof of the existence of smooth muscle in the walls of turtle heart, and the hearts of other species (except in blood vessels), although smooth muscle is demonstrable in the endocardium, but this plays no rôle in the functional activity of cardiac muscle.

8. Consequently, the seat of the depressant action of papaverin on the heart of turtle is not in the smooth muscle of the cardiac walls as alleged by Snyder and Andrus. This applies also to chelidonin and other alkaloids of the benzyloquinolin group.

9. The results of the observations in this report are also of value in appraising the importance of the cardiac factor in circulatory collapse resulting from the administration of these drugs or other causes, and their therapeutic value in collapse.

10. Accordingly, therefore, morphin would not be expected to cause cardiac collapse or injure the heart in collapse by direct action, while papaverin, chelidonin, the cotarnin salts, hydrastin and narcotin could cause cardiac collapse and injure a depressed heart.

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ATTEMPT TO DETECT THYROID SECRETION IN BLOOD OBTAINED FROM THE GLANDS OF INDIVIDUALS WITH EXOPHTHALMIC GOITER AND OTHER CONDITIONS INVOLVING THE THYROID

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The possibility of testing the hypothesis of augmented thyroid secretion in exophthalmic goiter and some other conditions involving the thyroid gland was suggested by the results reported by Rogoff (1) in which it was found that under the influence of stimulation of the cervical sympathetic and of massage of the gland the thyroid blood collected from a gland rich in colloid (and iodine) contained a sufficient amount of material to give the same effect on tadpoles as is caused by feeding very small amounts of the desiccated gland.

Reid Hunt (2) reported the results of some observations made with *systemic* blood, obtained at autopsy from a woman who had had exophthalmic goiter (also broncho-pneumonia and general peritonitis). He employed the "acetonitrile reaction." It is obvious that the conditions would be much more unfavorable for the detection of active thyroid material in the systemic blood collected *post mortem* than in *thyroid blood* collected during life.

Our experiments were made with thyroid bloods obtained from fifteen cases upon which thyroidectomy was performed.¹ The clinical diagnosis in ten of these cases was exophthalmic goiter, in three adenoma and two colloid goiter. Histological examinations of the glands revealed wide variations in their

¹ We are very much indebted to Dr. G. W. Crile for his courtesy in supplying the material for this investigation.

content of colloid, degree of hyperplasia, etc. Chemical examination for iodine indicated similar variations, corresponding closely with the colloid content of the gland, and ranging from no detectable iodine to 2.81 mgm. per gram of desiccated thyroid. We were thus able to compare the effect on tadpoles, of bloods from thyroids with large colloid and iodine contents, with that of bloods from thyroids with low colloid and iodine contents. The conditions were therefore favorable for the detection of an augmented secretion of thyroid active substance if such was present. Additional control comparisons were made in each case with systemic blood from the same individual that the thyroid blood was obtained. It must be pointed out that the large blood flows at the time of collection of the thyroid bloods, under the conditions of our experiment, were unfavorable for detecting active material in the blood because of the greater dilution of the material coming from the gland than was the case in the experiment reported by Rogoff.

The clinical and laboratory data are given in the accompanying table on following page.

METHOD

A specimen of thyroid blood was obtained at the time of operation by making a small cut in the thyroid vein. Systemic blood was obtained by venepuncture, in each case about a week after the operation, to be used as a control for the corresponding thyroid blood. The excised thyroid, after preserving a portion for histological examination, and the blood specimens were placed into a drying oven and desiccated at a temperature of 50° to 55°C. When completely dried, each of the gland and blood specimens was ground into a fine powder and preserved in a well stoppered container. Iodine determinations were made on all the thyroid specimens and on most of the thyroid bloods (when sufficient amount was available). No reaction for iodine was obtained with any of the blood specimens.

The tadpoles were fed in the same manner as described in previous papers (1), (3). As controls for the thyroid-fed tadpoles some were fed with dried lymph gland, some with dried liver, and some with fresh liver. In addition to feeding with the systemic bloods, which controlled each corresponding thyroid blood, some tadpoles were fed with dried

NUMBER OF CASE	AGE	CLINICAL DIAGNOSIS	DURATION OF SYMPTOMS	WEIGHT OF GLAND	IODINE PER GRAM OF DRIED GLAND	AMOUNT OF COLLOID IN HISTOLOGICAL SPECIMEN OF GLAND	HISTOLOGICAL DIAGNOSIS
	<i>years</i>			<i>grams</i>	<i>mgm.</i>		
1	39	Adenoma	Since puberty	230	0.92	Large	Diffuse colloid—adenomatous goiter
2	28	Adenoma	10 years	60	0.37	Moderate	Adenoma—foetal type
3	21	Colloid goiter	16 years	140	1.03	Large	Diffuse colloid—adenomatous goiter
4	37	Exophthalmic goiter	1 year	80	0.61	Moderate	Goiter—hyperplastic and hypertrophic moderate; foetal adenoma intermediate
5	38	Exophthalmic goiter	6 months	35	0.72	Moderate	Goiter—hyperplastic and hypertrophic mild
6	29	Exophthalmic goiter	8 months	80	0.07	Slight	Goiter—hypertrophic and hypertrophic marked
7	26	Exophthalmic goiter	1 year	35	2.60	Large	Goiter—hypertrophic and hypertrophic mild
8	29	Exophthalmic goiter		30	1.10	Small	Goiter—hypertrophic and hypertrophic marked
9	59	Adenoma	3 years	95	2.74	Large	Colloid adenoma—foetal type
10	19	Colloid goiter	6 years	230	0.00	None	Adenoma—foetal intermediate cystic (parenchyma destroyed)
11	24	Exophthalmic goiter	5 months	45	0.70	Moderate	Goiter—hypertrophic and hypertrophic mild
12	34	Exophthalmic goiter	4 months	80	0.86	Small	Goiter—hypertrophic and hypertrophic moderate
13	38	Exophthalmic goiter	11 months	115	1.14	Large	Goiter—hypertrophic and hypertrophic mild
14	28	Exophthalmic goiter	7 to 8 months	100	2.81	Large	Colloid goiter—foetal adenoma intermediate
15	43	Exophthalmic goiter	18 months	40	1.36	Large	Goiter—hypertrophic and hypertrophic mild—foetal adenoma intermediate, cystic

cattle blood. The activity of the thyroid gland specimens was approximately estimated by the method of bio-assay described by one of us (3). A previously standardized preparation of cattle thyroid was fed in doses ranging from 0.1 mgm. to 50 mgm. and the activity of each thyroid specimen was judged by comparison with the dose of the standard which yielded, as nearly as possible, the same effect as was caused by the amount of the specimen fed to the tadpoles. The comparison is made as soon as the standard indicates definite activity in the smallest doses employed.

Figure 1 shows the effect on tadpoles after the fourth dose of desiccated cattle thyroid had been given. The thyroid contained 2.15 mgm. of iodine per gram of dried gland. The numbers above the tadpoles indicate the amounts in milligrams of thyroid fed on alternating days, fresh liver being given on the other days. One tadpole was removed from each dish and sacrificed for photographing; the rest were carried on for further observation of activity until metamorphosis was nearly complete or death of most of the tadpoles in the dish occurred. The maximal effect was produced by 20 mgm. doses, at this stage, and the degree of angulation and emaciation is seen to be gradually less as the dose is smaller until only slight but definite angulation is seen with the 0.1 mgm. dose. At the same time and after the same number of doses of 20 mgm. each, the tadpoles receiving the desiccated glands from which the bloods were obtained were compared with the above set. The numbers above the tadpoles in figure 2, indicate the number of the case from which the gland was obtained. The control in figure 1 serves as the control for these tadpoles. It will be seen that gland 10 in which there was no detectable iodine (or colloid) caused only growth and that gland 6 with only a trace of iodine did not, at this stage, show as much activity as 0.1 mgm. of the standard cattle thyroid. All the other glands showed marked activity, but of course varying in degree. Two series of observations were made, one with tadpoles whose bodies were about 4 to 5 mm. in length when feeding was commenced, another with tadpoles about 6 to 8 mm. In the first series the dried bloods were offered in 75 mgm. doses and the thyroids in 20

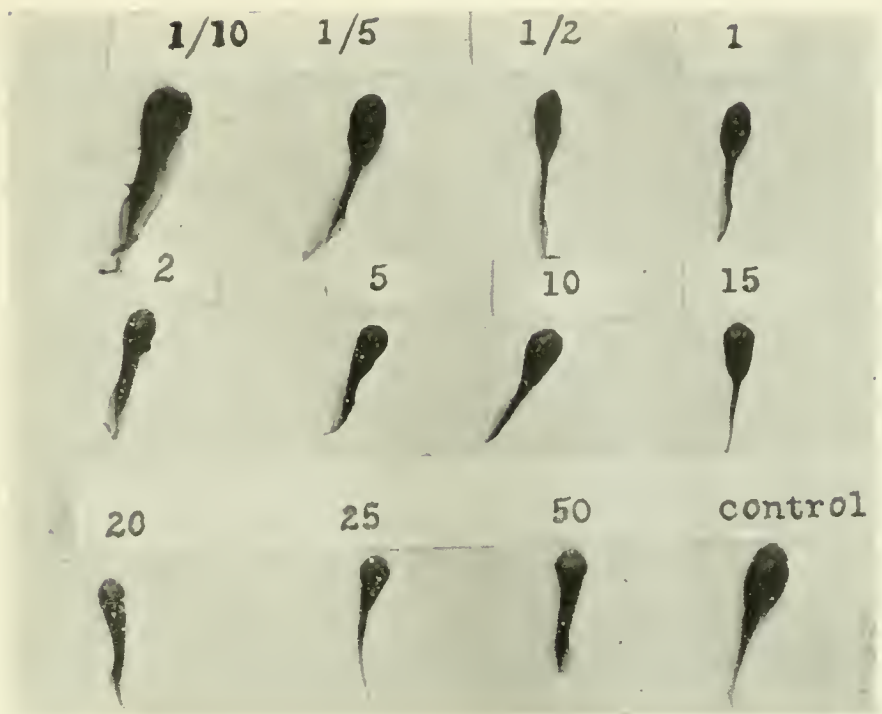


FIG. 1. SERIES I. TADPOLES AFTER FOUR DOSES RANGING FROM 0.1 MG. TO 50 MG. OF STANDARD DESICCATED CATTLE THYROID

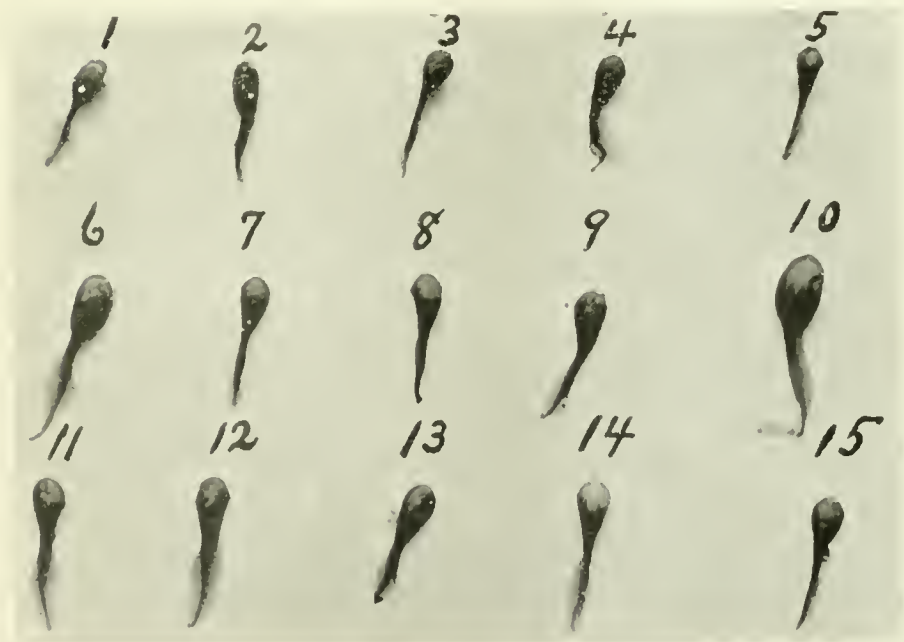


FIG. 2. SERIES I. TADPOLES AFTER FOUR 20 MG. DOSES OF DESICCATED THYROID

The numbers indicate the case from which the gland was obtained

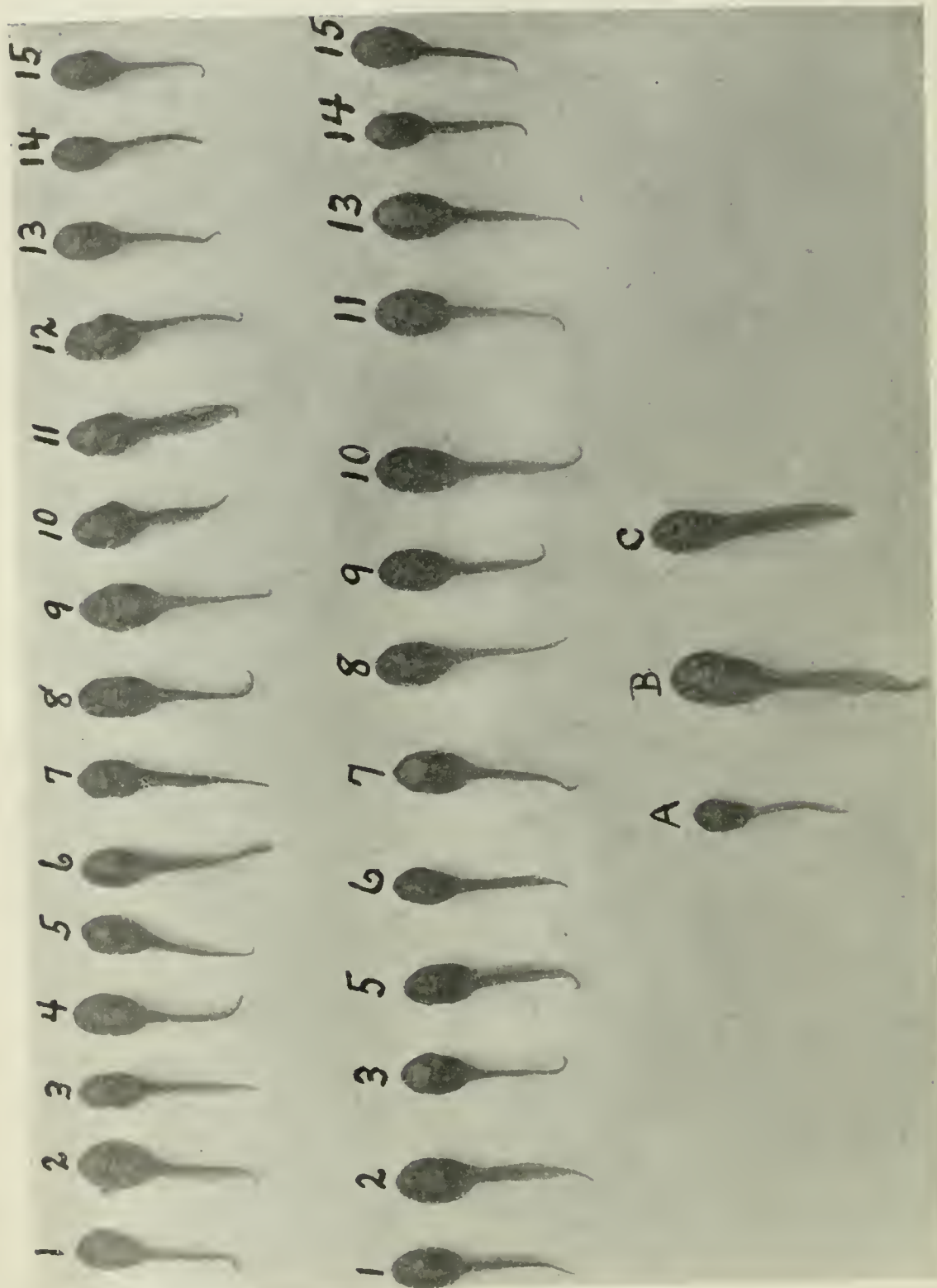


FIG. 3. SERIES II. TADPOLES FED WITH 100 MG. DOSES OF DRIED BLOOD EVERY OTHER DAY FROM JUNE 13 TO JULY 10

Upper row received thyroid blood, middle row corresponding systemic blood. A and C, controls; B, 100 mgm. doses of desiccated liver. The numbers indicate the case from which the blood was obtained.

mgm. doses given every other day. In the second series the bloods were given in 100 mgm. and the thyroids in 50 mgm. doses.

In figure 3 the upper row of tadpoles shows one of each set receiving 100 mgm. doses of dried thyroid blood on alternating days. The number over each tadpole corresponds with the numbers in figure 2 and indicates the gland from which each of these bloods was obtained. The middle row shows a sample of the tadpoles receiving the corresponding dried systemic blood in the same doses. Systemic blood was not obtained from cases 4 and 12. The controls for this set, A, received only fresh liver every other day. B, shows the effect on growth of substituting 100 mgm. doses of desiccated liver for fresh liver in one set of controls, and C, feeding of cattle blood in 100 mgm. doses.

No evidence was obtained, of activity characteristic of thyroid effect, from any of the thyroid bloods. Nor was there any detectable difference observed between the tadpoles fed on thyroid blood and those getting the corresponding systemic blood, or dried cattle blood. As has been previously mentioned, it is obvious that the high rate of blood flow, especially in glands which are enlarged (goiter), must result in considerable dilution of any active material secreted by the thyroid, so that if present its concentration in the blood might be below detectable limits.

The tadpoles receiving blood (human or cattle) grew much more rapidly than those getting only fresh liver on alternate days, or those getting dried lymph gland every other day and fresh liver on the alternate days. Still more rapid growth was seen in the tadpoles which were fed on desiccated liver instead of fresh liver in one control set.

SUMMARY

Blood was obtained from thyroid glands of ten individuals upon whom thyroidectomy was performed for exophthalmic goiter, three for thyroid adenoma and two colloid goiter, and tested for the presence of thyroid secretion, utilizing the "tadpole reaction." No evidence was obtained, in any of the specimens, of the presence of active thyroid material in the blood.

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THE LIVER AS A BLOOD CONCENTRATING ORGAN

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In a series of articles on acute polycythemia it has been shown by one of us (1, 2, 3, 4, 5) that the sudden increase in the number of red blood corpuscles per unit volume of blood takes place in the liver and that the process is one of blood concentration due to the escape of fluid into the liver lymphatics. It is the object of this paper to explain the mechanism in the liver by which epinephrin concentrates the blood.

The term "blood concentration" is inaccurate as the blood is not only a complex mixture but a suspension of formed elements in this mixture. The discussion of blood concentration and a review of the methods determining it, has been taken up in a recent paper by one of us (6) to which the reader is referred for details. For our present purpose the amount of hemoglobin per unit volume of blood although inaccurate will suffice to give us an index of variations in the fluid content of the blood.

In order to accentuate changes of blood concentration a fluid was injected in the form of isotonic salt solution. It has long been known that isotonic salt solution rapidly leaves the circulation on account of the increased partial pressure of the salt. Due to the absence of colloids, the salt will have a tendency to diffuse into the tissues carrying water with it. If however, gum acacia is added, to take the place of the blood colloids, this gum salt solution will remain in the vessels for hours as shown recently by Bayliss (7). In order to determine if there is a special mechanism in the liver for the removal of this fluid, the following experiments were performed.

EXPERIMENTAL DATA

Twenty-five cubic centimeters of 0.9 per cent sodium chloride solution was injected into the saphenous vein of dogs, by means of a modified Woodyatt pump at such a rate that the injection occupied ten to eleven minutes. A constant time of injection, instead of rate, was chosen in order that the results might be directly comparable. The dogs were anesthetized with ether in order to insure uniform conditions and maintain a constant concentration of the blood. It may be pointed out here that this is necessary in dogs and cats as the concentration of their blood, without anesthesia, is constantly changing in untrained animals due to emotional stimuli. This was pointed out by Lamson in 1915 (1), for which he has been recently criticized by Smith and Mendel (8), who tested out the constancy of blood concentration under similar conditions and found no variation in the number of erythrocytes. They made the mistake, however, of using rabbits, instead of dogs apparently not noting that the above mentioned author has shown that it is impossible to vary the number of erythrocytes per unit volume of blood in the rabbit, by any of the usual methods of emotional disturbances, the injection of epinephrin, etc. (2). As an index of blood concentration, the hemoglobin was determined by withdrawing 0.25 cc. of blood, by means of a small hypodermic syringe, from the exposed jugular vein, diluting with 0.04 per cent ammonia and saturating with carbon monoxide. The first sample was taken as a standard and the others compared with it in the Dubosq colorimeter. The samples were first collected, then diluted and read at one time, so that there were very slight chances of change in the standard.

NORMAL DOGS

Eight normal dogs showed a great constancy in the hemoglobin curve after the salt injection. The average time for the return of the hemoglobin to normal concentration was thirty-three minutes. The maximum variation plus or minus three minutes.

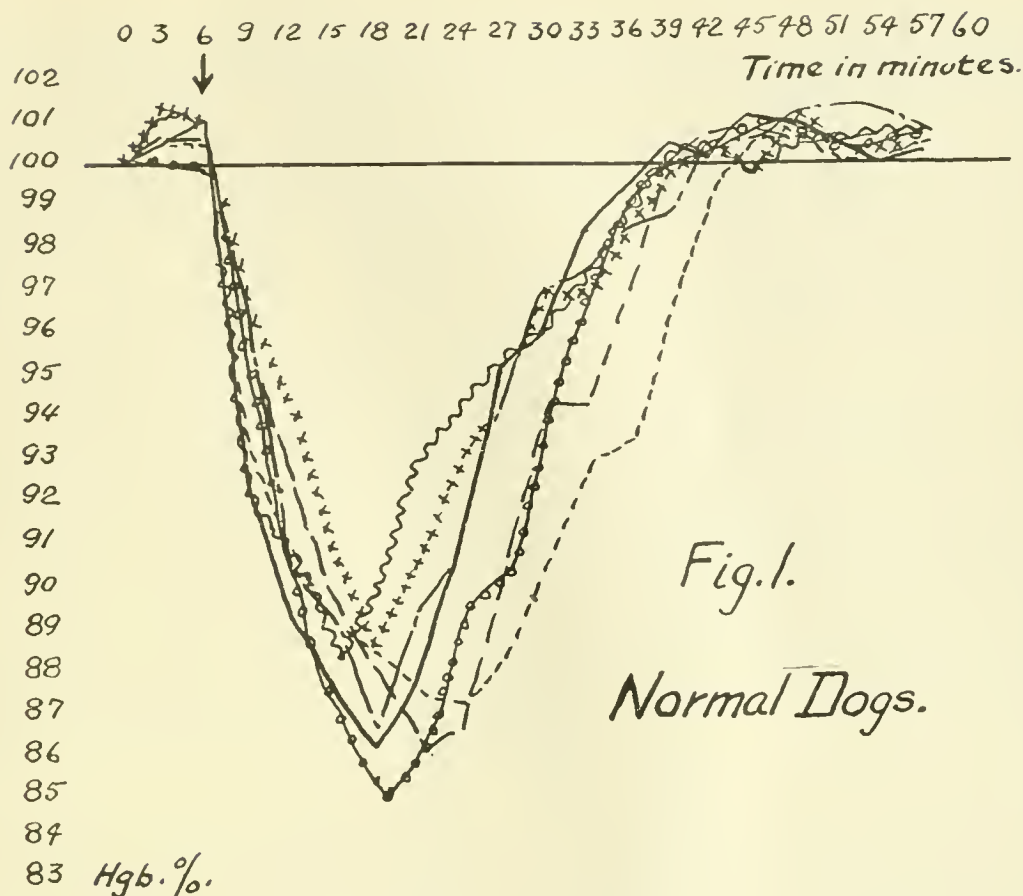


FIG. 1. SHOWING THE DEPRESSION OF THE HEMOGLOBIN CURVE AFTER THE INJECTION IN TEN TO ELEVEN MINUTES OF 25 CC. ISOTONIC SALINE PER KILOGRAM IN EIGHT NORMAL DOGS

DOGS WITH LIVER REMOVED

The dogs were anesthetized with ether and an Eck fistula made between the portal vein and vena cava, using a three-legged clamp and an uninterrupted blood flow. The portal vein was tied at its entrance to the liver, the hepatic artery was also tied and severed, thus cutting off the liver from all blood supply. The salt solution was injected as before, *except only four-fifths of the amount was used*, allowing one-fifth to approximate roughly the amount of blood held by the liver. This was done in order that an increase in the time taken for the return of the hemoglobin curve to normal could not be ascribed to the injection of the same amount of fluid into a circulatory system of decreased capacity. Two dogs were used, the average time taken for the

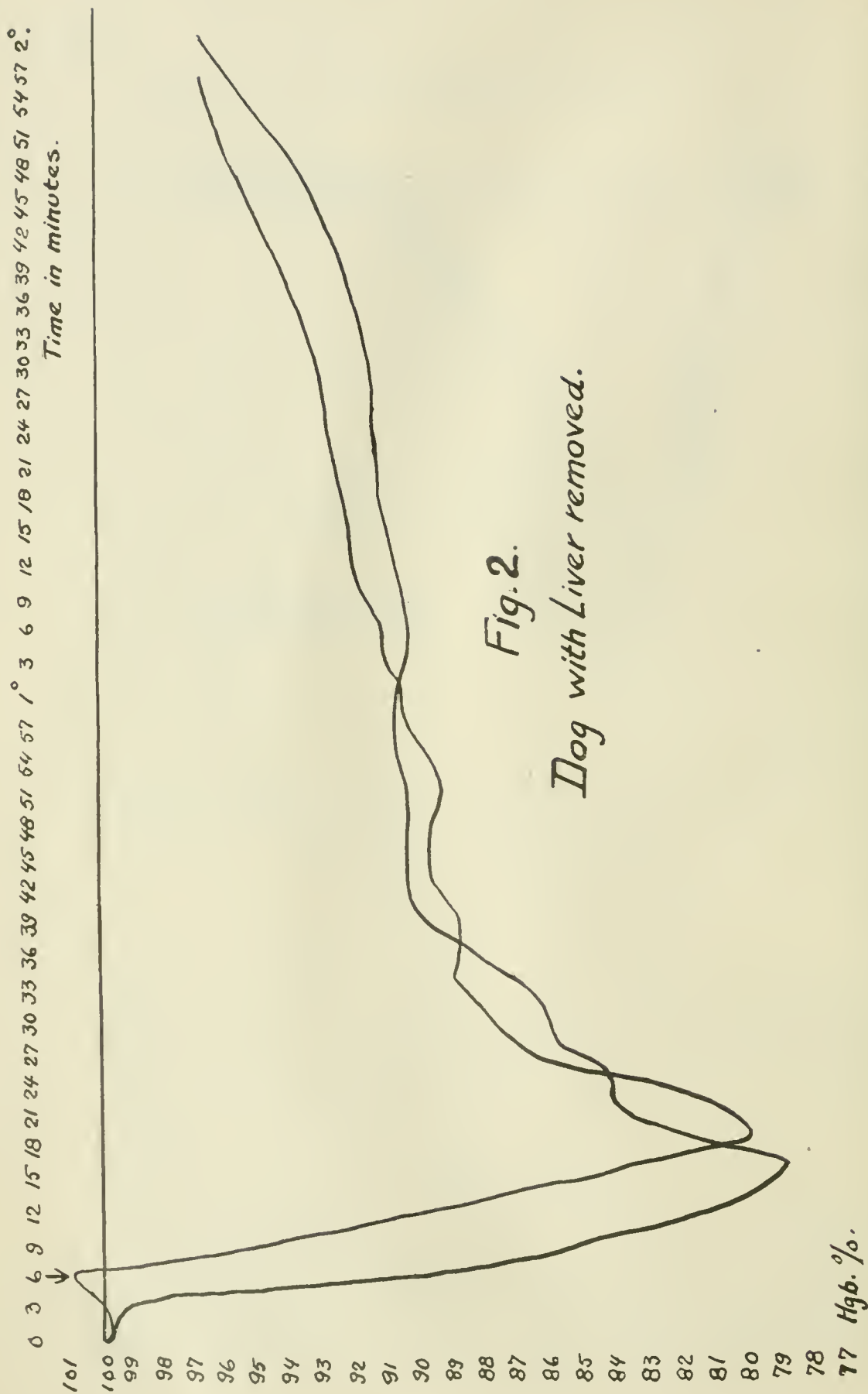


FIG. 2. SHOWING THE DEPRESSION OF THE HEMOGLOBIN CURVE IN TWO DOGS IN WHICH THE LIVER HAS BEEN REMOVED FROM THE CIRCULATION

Twenty cubic centimeters of isotonic saline per kilogram was injected in ten to eleven minutes. The great increase in time taken for the return of the hemoglobin curve to normal will be noticed.

hemoglobin curve to return to normal was two hours, or approximately four or five times as long as in the normal dog. It will be noticed that the depression of the curve is roughly that in the normal animal, showing that the approximation of the decreased volume of the circulation by removal of the liver, was essentially correct. The first part of the upward curve is very similar to that in the normal animal, but then the curve flattens out returning very slowly to normal.

A comparison of these two investigations shows definitely that the liver plays an important part in the removal of the injected salt solution. One might consider that this action of the liver is merely due to the great surface of its capillaries and that it consequently plays a large part in the same process of diffusion going on in the rest of the body. In order to show that some other process takes place in the liver besides this, the following experiments were undertaken.

NORMAL DOGS WITH EPINEPHRIN

The experiments were carried out exactly as before, except that 0.9 mgm. per kilo of epinephrin was added to the injection fluid. The following curve shows the result of this procedure. Two

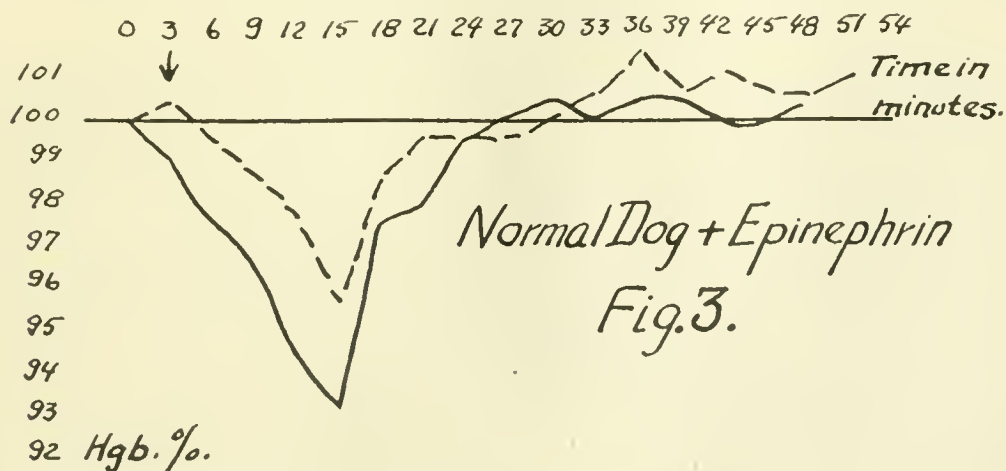


FIG. 3. SHOWING THE DEPRESSION OF THE HEMOGLOBIN CURVE IN TWO NORMAL DOGS AFTER THE INJECTION IN TEN TO ELEVEN MINUTES OF 25 CC. ISOTONIC SALINE PER KILOGRAM TO WHICH 0.9 MG. PER KILOGRAM OF EPINEPHRIN HAS BEEN ADDED

The very slight depression of the curve, and the very rapid return to practically normal will be noted. (The typical blood concentration with epinephrin.)

dogs were used, the average time taken for return of the hemoglobin curve to normal was twenty-seven minutes. It will be noticed that not only was the time taken for the disappearance of the fluid about half that when the salt solution alone was injected, but the depression of the curve was extremely small, showing that the fluid left the blood almost as quickly as it was injected.

DOG WITHOUT LIVER AND EPINEPHRIN

The liver was removed from the circulation as in the above experiment, the injection was made as before. *Only four-fifths of the amount of salt solution used in the normal animal was injected*, to this was added 0.9 mgm. of epinephrin per kilo. Two dogs were used, and the hemoglobin had not returned to normal in either case after two hours. It will be noticed that this curve is practically the same as that obtained when normal salt solution was injected into dogs, without the liver. This experiment shows that epinephrin although acting on the entire body, with the exception of the liver, in no way influences the rate at which the injected salt solution leaves the circulation, when the liver is removed.

LIVER VOLUME EXPERIMENT

In order to show that fluid is taken up by the liver, dogs were placed under ether and salt solution intravenously injected as in the other experiments. Through the kindness of Dr. Eben C. Hill of the department of Anatomy of this University, the change in liver volume was shown by a series of instantaneous Roentgenograms, taken at intervals during the injection. The increase in size of the liver was quite evident, but in order to be certain of this small brass clips were attached to the inferior edge of the liver and to the tips of the lobes. Twenty minutes after the injection, of 25 cc. of isotonic saline per kilo, a downward and lateral change of position of between 1 and 2 cm. was observed, showing definitely an increase in volume of the liver.

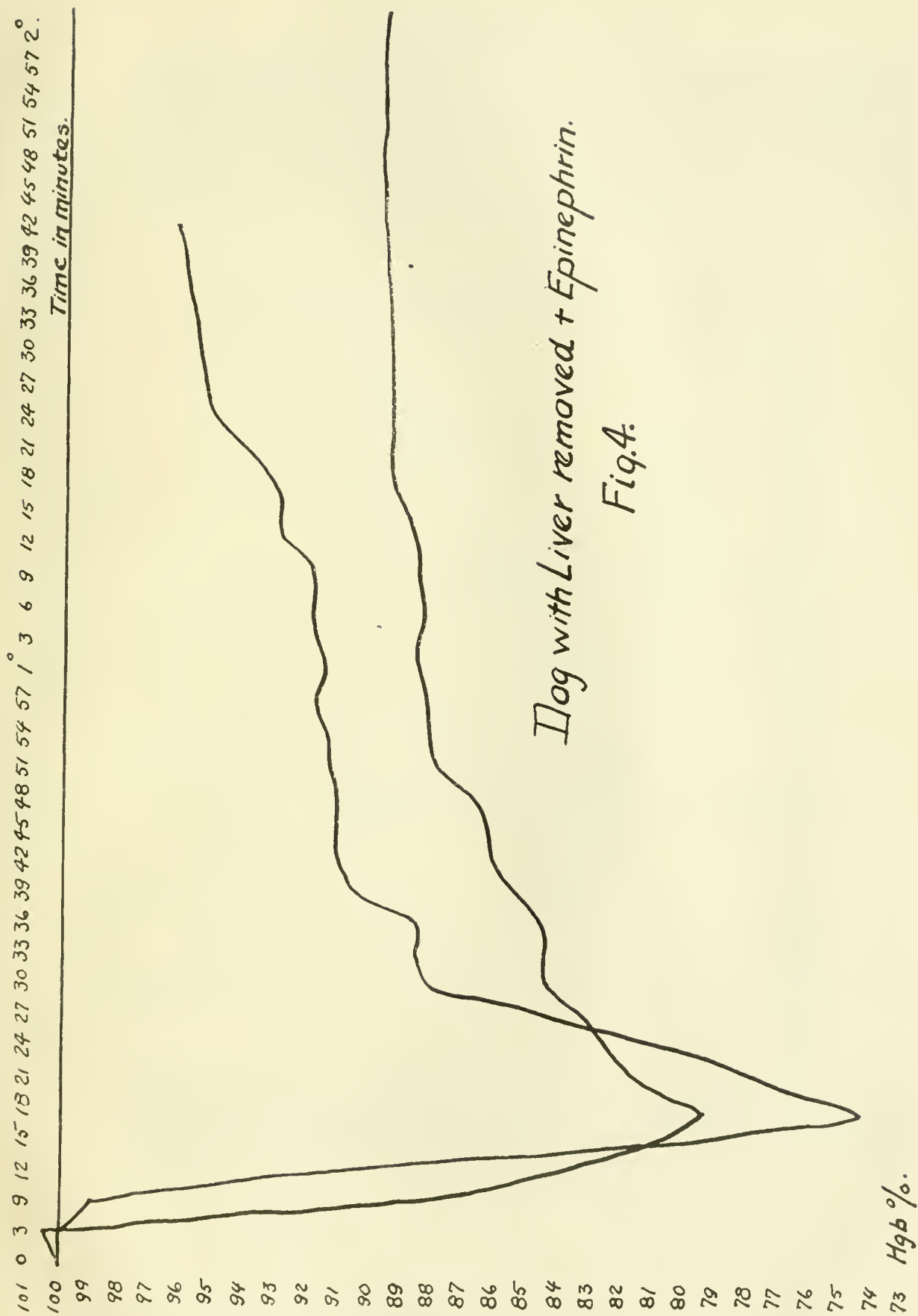


FIG. 4. TWENTY CUBIC CENTIMETERS PER KILOGRAM OF ISOTONIC SALINE PLUS 0.9 MG. OF EPINEPHRIN WERE INJECTED INTO TWO DOGS AFTER THE REMOVAL OF THE LIVER FROM THE CIRCULATION
It will be noticed that the addition of epinephrin in no way influenced the rate of disappearance of the salt solution after the removal of the liver.

RABBIT EXPERIMENTS

Rabbits were placed under ether and the injection of salt solution carried out as in the other experiments. Three rabbits were used, the hemoglobin curve returning to normal in an average of 12 minutes. The rate of fluid loss in the rabbit is seen to be much more rapid than in the dog.

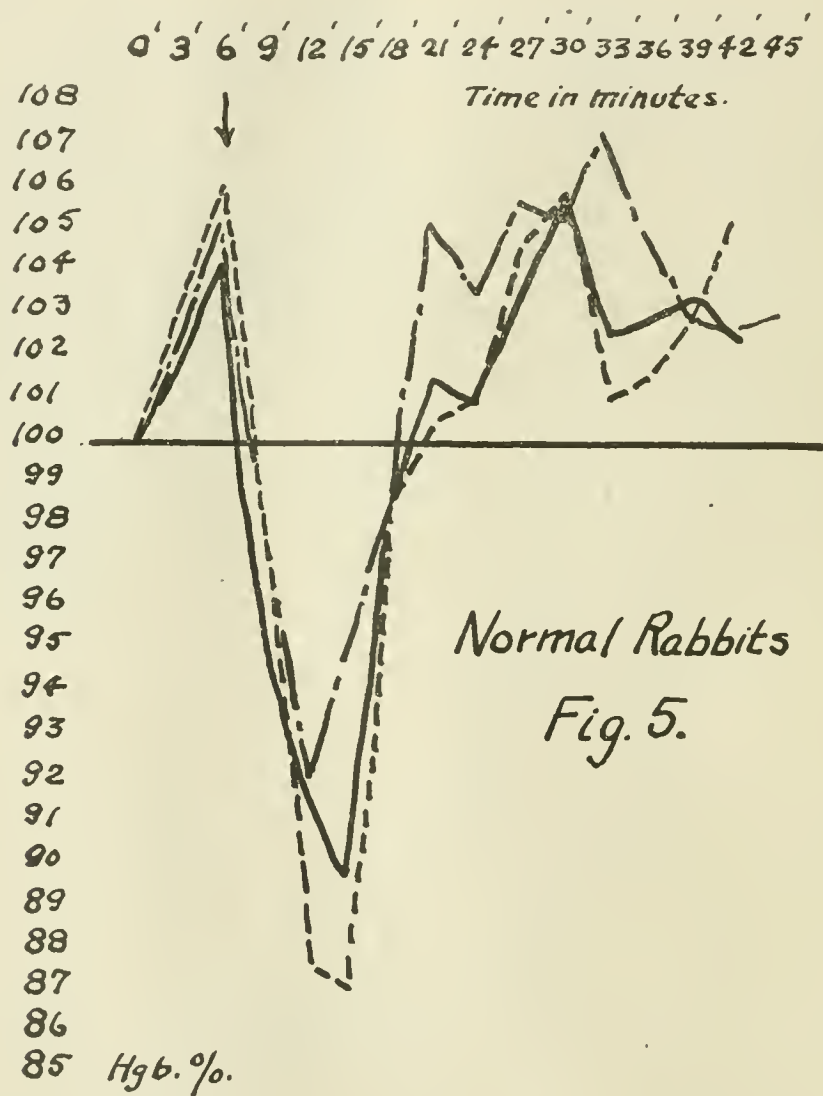


FIG. 5. TWENTY-FIVE CUBIC CENTIMETERS PER KILOGRAM OF ISOTONIC SALINE WAS INJECTED INTO THREE RABBITS SHOWING THE DEPRESSION OF THE HEMOGLOBIN CURVE

RABBIT AND EPINEPHRIN

The addition of epinephrin to the injection fluid caused the rate of disappearance to increase to such a degree that the fluid left the blood stream more quickly than it entered, giving an increase in the hemoglobin curve instead of a decrease, showing a concentration of the blood in three rabbits.

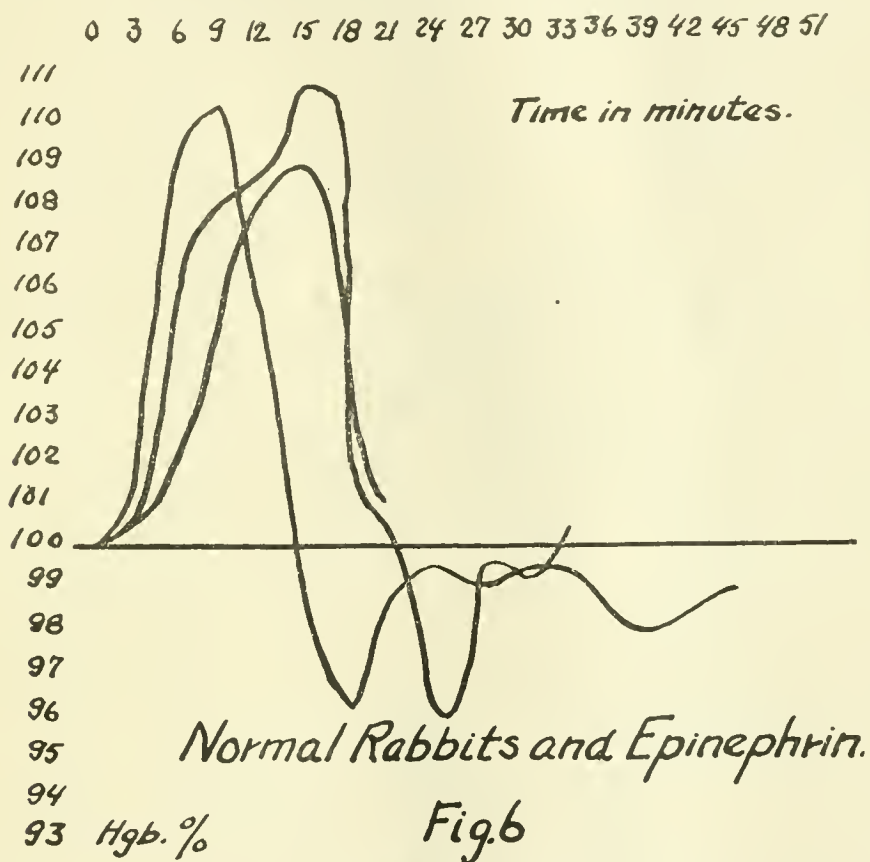


FIG. 6. TWENTY-FIVE CUBIC CENTIMETERS OF ISOTONIC SALINE PLUS 0.9 MG. OF EPINEPHRIN PER KILOGRAM WAS INJECTED INTO THREE RABBITS

These all showed a *concentration* of the blood instead of a dilution. It is of interest to note that epinephrin alone causes no concentration of the blood.

It is hoped in the near future to remove the rabbit's liver and determine the rate of fluid loss under these conditions, but at present on account of the difficulty of technique, this has not been done.

DISCUSSION

These experiments show that isotonic saline leaves the circulation four or five times as rapidly in the normal animal as in one in which the liver has been removed from the circulation. They also show that epinephrin greatly increases the rate at which isotonic saline leaves the circulation in the normal animal, but in no way influences the rate in an animal in which the liver has been removed. Finally it has been shown that coincident with the disappearance of the fluid from the circulation, there is a swelling of the liver. From these facts and others collected in previous experiments we are in a position to explain the process taking place in the liver, by which the blood is concentrated.

The circulation may be represented diagrammatically as in figure 7, divided into the portal and systemic circulations. If epinephrin be given in large doses the following will occur, the arterial pressure will be raised, the portal pressure will be raised but the cava pressure will remain constant (9). This shows an obstruction in the liver, for an obstruction higher up would have caused an increase in the cava pressure also. Coincident with the increase in portal pressure there is a swelling of the liver (10), a concentration of the blood and after a considerable latent period a greatly increased flow of lymph from the thoracic duct which is shown to come from the liver (11).

The cause of this obstruction to the flow of portal blood through the liver might occur from constriction of the portal vein and its branches, constriction of the hepatic veins, or a swelling of the liver cells blocking the lumen of the vessels. It has been shown by Bainbridge and Trevan (12) that the injection of distilled water into the portal vein caused an immediate increase in portal pressure which they felt was due to swelling of the liver cells. They suggested that the action of epinephrin in the liver might liberate sugar from glycogen causing an increased osmotic pressure and a swelling of these cells by the taking up of water which would in turn press on the lumen of the vessels and cause obstruction to flow. As epinephrin causes no obstruction to the blood flow in the rabbit's liver although

Furthermore reference to the diagram will show that any obstruction to the portal vein will not increase the filtration pressure in the liver, but in the other portal organs. Finally if the liver is removed from the circulation by means of an Eck fistula and tying the hepatic artery, epinephrin does not cause a sufficient

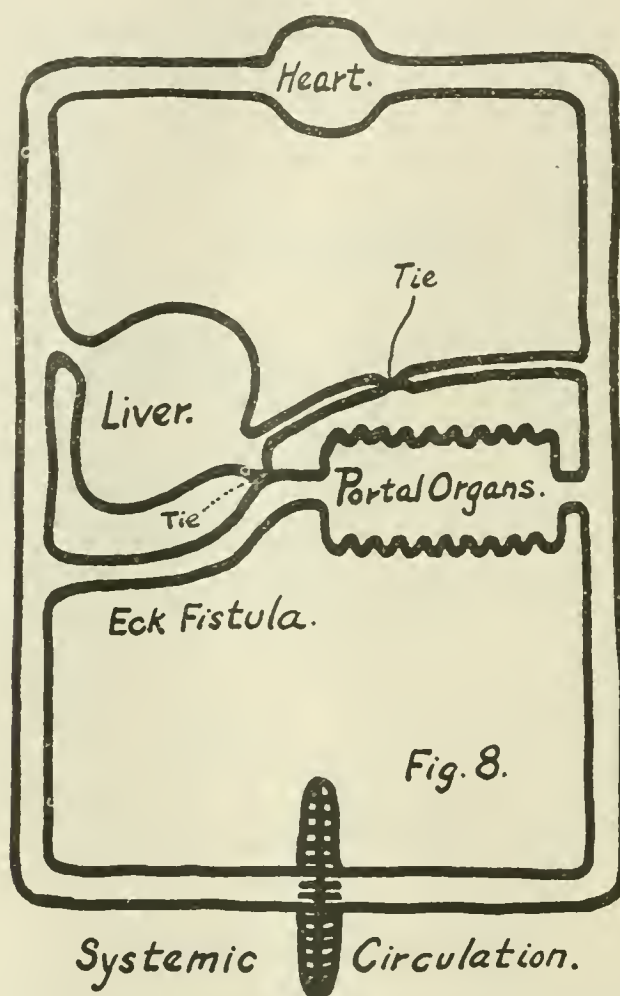


FIG. 8. IF THE LIVER IS REMOVED FROM THE CIRCULATION AND THE PORTAL BLOOD SHUNTED AROUND IT BY MEANS OF AN ECK FISTULA IT WILL BE SEEN THAT THERE IS NO OBSTRUCTION TO THE VENOUS RETURN IN THE ENTIRE BODY

constriction of the extra-hepatic portal branches to cause back pressure in the other organs and loss of fluid as shown by no change in the blood concentration, after the injection of epinephrin in such an animal (4). *Portal constriction is not then the cause of this obstruction to the blood flow in the liver, but the obstruction must occur on the distal side of the hepatic capillaries.*

Constriction of the hepatic veins. After an Eck fistula is done and the hepatic artery tied off as in figure 8 it will be seen that there is no obstruction to the venous return in the whole body. Epinephrin injected into such an animal greatly increases the arterial pressure but does not increase the venous pressure, therefore, there is no increased filtration pressure and no loss of fluid into the tissues as shown in the above experiments. After epinephrin is injected into a normal dog, however, an obstruction to blood flow occurs in the liver. If the obstruction were in the portal vein or its branches it would be on the proximal side of the liver capillaries and no increased filtration would take place in the liver, but the liver swells, therefore, this is not the case.

Furthermore, after the removal of the portal organs, if the hepatic artery is left intact, epinephrin causes the same concentration of the blood and swelling of the liver. As there is no portal circulation in this case, as arterial constriction alone has no influence on blood concentration, as fluid is lost from the blood, and as the liver swells and the red count is increased as in the normal animal, we may conclude that an obstruction to the blood flow, has occurred in the liver, and the only place where such an obstruction could occur and cause the above changes would be in the hepatic veins.

It was shown by one of us (2) that epinephrin causes no concentration of the blood in the rabbit, but does so in the dog. Mautner and Pick (17) have shown that the dog's liver vessels constrict with epinephrin while the rabbit's do not and it is seen here that the obstruction in the liver must take place in the hepatic veins. This causes increased filtration pressure and fluid loss, therefore, the dog's hepatic veins must differ from those of the rabbit and also from the portal veins. On consulting the anatomists to see if such a difference had been observed, we were referred to the recent work of Arey and Simonds (19) who following the work of Maunter and Pick on peptone shock examined the hepatic vessels of the dog and rabbit and found that the dog's hepatic veins, are equipped with very strong muscles

while those of the rabbit and the portal veins of each animal possess very little if any musculature.

We find then in the liver of the dog the following mechanism by which the blood may be suddenly concentrated; a constriction of the hepatic veins, an obstruction to the flow of blood through the liver, an increased pressure in the portal veins and hepatic artery, an increased filtration pressure in the liver capillaries, filtration of fluid into the liver lymphatics causing a swelling of the liver and after a considerable latent period a gradual return of fluid to the circulation via the thoracic duct. The hepatic veins thus occupy a unique position in the circulation, being the only mechanism in the body which can cause an obstruction to the venous return and thus cause an increased filtration pressure in the capillaries. Fluid may be suddenly lost from the body by any of the channels of excretion, fluid may be taken up by the tissues in disease from pathological changes of the salt concentration in the tissues or blood, but the mechanism in the liver is the only one by which fluid may be suddenly lost from the circulation but not from the body.¹

These experiments show conclusively that the criticism of our previous work on polycythaemia, by Scott (19) is unjustifiable. He sought to prove that the liver played no part in polycythaemia but that any increase in blood pressure causes a general loss of fluid into the tissues and a decrease, the reverse.

The question of a nervous venous control of this mechanism is still undecided. It was shown by one of us (1) that a very great concentration of the blood occurs in emotional disturbances in the dog and cat and since none takes place in the rabbit, this would indicate a nervous control of this same mechanism in the liver. Fright has however been shown to cause the output of epinephrin (20) and this substance may act upon the hepatic veins rather than there being a direct nervous stimulation of the hepatic plexus. Stimulation of the hepatic artery, the portal vein and the sympathetics, by the present authors and

¹ Bronchial constriction, cardiac failure, etc., may cause increased intrapulmonary pressure and the filtration of fluid into the alveoli, but this is a pathological and not physiological process.

various investigators, in no case can approximate the changes in blood concentration and portal pressure occurring after the injection of epinephrin and even the injection of epinephrin cannot cause as great an increase as emotional disturbances, yet the constriction following epinephrin would indicate sympathetic terminals in these vessels.

Burton-Opitz (21) by nerve stimulation was able to show strong vasomotor nerves in the hepatic artery and very weak ones in the portal vein. On injecting minute doses of epinephrin into the portal vein he obtained slight obstruction to the portal flow and none to the flow of blood from the hepatic artery. If larger amounts were injected into either vessel obstruction to flow occurred in both systems from which he concluded that "it has also been established that the portal mechanism is distinct from the one located in the terminals of the hepatic artery, and it embraces not only the distributing tubules but also an extensive area of the central or collecting channels." The reflex paths by which fright, asphyxia, etc., cause concentration of the blood is still to be worked out.

In the experiments here reported there was a great difference in rate of disappearance of saline in normal dogs and in those without the liver. The action of the liver in this case may be due simply to the great capillary surface from which saline could diffuse, to a reflex action of the constrictor mechanism described above, or to a swelling of liver cells caused by the absorption of salt solution and consequently causing an obstruction to blood flow and an increased portal pressure. Such an obstruction to flow has been shown by Bainbridge and Trevan when distilled water was injected into the portal vein.

CONCLUSIONS

1. The disappearance of intravenously injected isotonic salt solution from the blood is due in large part to the action of the liver. The rate of disappearance is decreased four times or more by the removal of the liver.

2. The addition of epinephrin to the injected fluid greatly increases its rate of disappearance in the normal animal but has

no effect in an animal whose liver has been removed from the circulation.

3. The liver occupies a unique position in the circulation on account of being the only organ in the body having a constrictor mechanism on the *venous side of its capillaries*. This powerful constrictor mechanism in the hepatic veins of certain species of animals as the dog, is acted upon by epinephrin and is under nervous control. Obstruction to the hepatic blood flow by constriction of the hepatic veins causes an increased filtration pressure throughout the enormous area of liver capillaries, the filtration of the fluid into the liver lymphatics, a concentration of the blood and an increase in the number of red blood corpuscles per unit volume of blood. After a considerable latent period this fluid is returned to the blood stream by way of the thoracic duct.

4. The theory that any increase in arterial pressure will cause a general filtration of fluid into the tissues is incorrect.

5. The addition of epinephrin to intravenous salt infusion for the purpose of raising the blood pressure should be discouraged as it accelerates fluid loss.

6. The acute polycythaemia found in emotional disturbances, asphyxia, exercise and after the injection of certain drugs can all be explained by this mechanism, but as yet it is not proven that they do occur in this manner only.

7. Fluid may be lost from the body by any of the channels of excretion. It may leave the blood stream by diffusion in certain pathological conditions but this mechanism in the liver is the only known mechanical device by which fluid may be removed from the circulation by filtration and yet not be lost from the body.²

² Obstruction to the pulmonary circulation can take place, but the filtration of fluid into the lungs cannot be considered a physiological condition.

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